The priming effect of rewards and the role of dopamine transmission

Czarina Evangelista

A Thesis
In the Department
Of
Psychology

Presented in Partial Fulfilment of the Requirements
For the Degree of
Doctor of Philosophy (Psychology) at
Concordia University
Montreal, Quebec, Canada

September 2019

CONCORDIA UNIVERSITY
SCHOOL OF GRADUATE STUDIES

This is to certify that the thesis was prepared

By: Czarina Evangelista

Entitled: The priming effect of rewards and the role of dopamine transmission

and submitted in partial fulfillment of the requirements for the degree of

Doctor of Philosophy (Psychology)

complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

Signed by the final Examining Committee:

_____ Chair
Dr. Saurabh Maiti

_____ External Examiner
Dr. James R. Stellar

_____ External to Program Examiner
Dr. James W. A. Grant

_____ Internal to Program Examiner
Dr. Uri Shalev

_____ Thesis Co-Supervisor
Dr. Wayne G. Brake

_____ Thesis Co-Supervisor
Dr. Peter Shizgal

Approved by _____
Dr. Andrew C. Chapman, Graduate Program Director

November 13, 2019 _____
Dr. Andre Roy, Dean of Faculty of Arts and Science

ABSTRACT

The priming effect of rewards and the role of dopamine transmission

Czarina Evangelista, Ph.D.

Concordia University, 2019

After receiving a reward, motivation to obtain more is boosted. For example, a taste of chocolate drives me to want and consume more chocolate—sometimes to the point that I finish an entire bar! This phenomenon is called the *priming effect of rewards*. The priming effect of rewards has primarily been studied with electrical brain stimulation. Rats primed with brain stimulation have been shown to prefer brain stimulation over competing rewards. Additionally, they work harder for more rewarding brain stimulation. Although over half a century of research implicates dopamine transmission in reward and motivation, the priming effect may not depend on dopamine transmission.

This thesis investigated the priming effect of electrical brain stimulation and food and the role of dopamine transmission. First, expanding on the original work on the priming effect of electrical brain stimulation, we examined whether the priming effect depends on the strength and cost of reward. We showed that the priming effect of electrical brain stimulation is more likely to be observed when the reward intensity is high and the cost is low. Secondly, we investigated whether the priming effect generalizes to other rewards such as food. We demonstrated that food also elicits a priming effect. Lastly, it was studied whether dopamine transmission is necessary for the priming effect of electrical brain stimulation and food. We showed that the priming effect of those rewards persists following dopamine receptor antagonism.

Although dopamine transmission is important for reward and motivation, the present thesis provides evidence that it may not be essential for the priming effect. This emphasizes the need to reconsider and investigate the role of non-dopamine systems in reward and motivation.

ACKNOWLEDGEMENTS

First and foremost, I want to thank my PhD thesis co-supervisors. Dr. Peter Shizgal, thank you for your guidance and for sharing your wisdom with me. The challenges we faced from the electrical priming experiments taught me patience, persistence, and resilience. There were many instances when I was discouraged by our results, but your positivity was contagious and it kept me going. Thank you for your invaluable help with data analyses and writing, and your willingness to meet on the weekends to work on revisions. Your guidance has helped me become a better critical thinker and researcher, and I will continue to strive to improve. Dr. Wayne G. Brake, thank you for seeing the potential in me when I was an undergraduate student from Dr. Melissa J. Glenn's lab. Whenever we reached road bumps with the priming experiments, you always managed to assure me that things will work out. Your pep talks really helped me stay motivated when the going got tough. Thank you for taking the time to sit with me to improve my writing, for instilling that voice that says "What would Wayne say?", and for insisting we meet regularly to make sure that I continue to make progress in my writing. Your support and guidance gave me more confidence as a speaker, scientist, and person. I consider myself immensely lucky to have had not one but two great mentors during my graduate studies at the CSBN. I truly cannot thank you both enough for all that you have done to help me get to where I am now.

Second of all, thank you to Dr. Uri Shalev, Dr. James W.A. Grant and Dr. James R. Stellar for agreeing to be members of my examining committee. I appreciate you all taking the time to read my thesis and for asking the hard questions. You have all helped me become a better writer, critical thinker, and scientist. Thank you for being part of this momentous journey.

The CSBN staff has provided me with tremendous support. Thank you to Isabelle Bouvier and Kim Breux for your help with contracts, registration, funding applications—and thanks for listening to me talk about my cats! Thank you to Heshmat Rajabi for your help with lab orders, assistance with lab equipment, forcing me to eat lunch outside on beautiful summer afternoons, and providing advice on my future endeavors. Thank you to Barry Robinson for helping with safety protocols and lab equipment. Last but not least, thank you to Dave Munro and Stephen Cabilio for your help with the operant boxes, the runway, and the Pref3 system. Without your help, I would not have been able to run my experiments and finish this thesis!

I want to thank all the undergraduate honors students, specialization students, and volunteers that worked with me. Thank you to Julia Pinheiro-Carvalho, Esthelle Ewusi-Boisvert, and Norhan Mehrez for your help with pilot studies and Chapter 2 experiments. Thank you to Michael Pillegi, Jacques, R. Voisard, Vanessa Boulos, Noemie Tito, Ramela Arax Koumrouyan, Smita Patel, Yaman Al-qadri, Brunella V. Gonzalez Cautela, Fei Xiang Zhou, Jesse Duchemin, Andrew Habrich, Victoria Lorenc, Collin Gagne, Khaoula El Oufi for assisting with Chapter 4 experiments. And thank you to the many other volunteers that contributed to a project in one form or another such as Matilda Lucas and Jasmine Pitsilis.

Thank you to the past and present Shizgal and Brake lab members. Thank you to my current lab members Smita Patel, Collin Gagne, Jesse Lacasse, and Vasilios Pallikaras for your support and entertainment, especially during the final stages of my thesis. Thank you to Allison Martel for teaching me how to make electrodes, how to construct electrical leads, and for your pilot work on the electrical priming study. Thank you Sarah Nolan-Poupart, Dr. Marie-Pierre Cossette, and Dr. Ivan Trujillo-Pisanty for helping me get acquainted with the lab when I first started, for teaching me how to perform surgeries for BSR, and for being one of my first group of friends at the CSBN. Marie-Pierre and Ivan, thank you for sharing your knowledge on how to conduct optogenetic experiments, how to manage a breeding colony, and how to perform genotyping. Milan Valyear, an honorary Shizgal lab/journal club member, thank you for also training me on how to breed THCre rats and how to genotype. Lastly, thank you to Dr. Waqqas Shams for helping me with literally anything—from placing ear bars, data analysis, funding applications, creating *dope* images on Keynote, and even giving me brief motivational speeches every now and then!

I want to thank all the friends I made at the CSBN. In particular, thank you to Franca Lacroix, Pavel Solis, Mandy Lecocq, Sarah Vehar, Christine Gerson, Dr. Nuria de Zavalia, Dr. Belinda Lay, Milan Valyear, and Franz Villaruel. I don't have the space nor time to recall all the great memories I've shared with these magnificent people, but they really put the fun in graduate school. The first year of graduate school was the hardest for me because I had just moved to a new city and new country where I had zero friends. I'm immensely grateful that I met each of you. I truly appreciate your company and friendship.

I want to thank my family for their endless support. Thank you to parents, Alma Evangelista and Robin Carreon. I know it has been hard having me live so far away from home,

but you have always been understanding, patient, and supportive of my work. I can't thank you enough for the sacrifices you made for me to be where I am now. I promise that I will continue to work hard to make you happy and proud. Thank you to my partner, Ariel Batallán. I am grateful that I was able to undergo my PhD journey with your support. Thank you for cooking when I was too tired, for advising me to hug a cat when I would cry from stress and anxiety, and for encouraging me to realize I am more capable than I give myself credit. Lastly, I have to thank my three cats: Nona, Rwby, and Didi. They won't be able to read this because they are cats… But I want to thank them for enduring my hugs, for laying on my laptop or my feet to force me to stop doing work and relax, and for simply being the silly cats that bring me an exorbitant amount of joy.

During my graduate studies, I received funding from FRQNT, Faculty of Arts and Science Graduate Fellowship, Concordia University Team Seed Grant, Concordia Merit Scholarship, FRQS/CSBN Entry Stipend, Carolyn and Richard Renaud Teaching Assistantship Scholarship, and Catherine and Robert Juster Graduate Student Scholarship. Your generosity made it possible for me to successfully complete my graduate studies.

CONTRIBUTION OF AUTHORS

Chapter 1: The general introduction was written by Czarina Evangelista and revised by Dr. Wayne G. Brake and Dr. Peter Shizgal.

Chapter 2: These experiments were designed by Czarina Evangelista, Dr. Wayne G. Brake, and Dr. Peter Shizgal. Experiment 1 was conducted by Czarina Evangelista. The MatLab code for analyzing the experiment 1 data was written by Dr. Kent Conover. Experiments 2 and 3 were conducted by Czarina Evangelista and Norhan Mehrez. The MatLab code for analyzing data from experiments 2 and 3 was written by Dr. Peter Shizgal. The findings were interpreted by Czarina Evangelista, Norhan Mehrez, Dr. Wayne G. Brake, and Dr. Peter Shizgal.

Chapter 3: These experiments were conceived and designed by Czarina Evangelista, Dr. Wayne G. Brake, and Dr. Peter Shizgal. Experiments 1 and 2 were conducted by Czarina Evangelista. The MatLab code for analyzing data from experiments 2 and 3 was written by Dr. Peter Shizgal. The findings were interpreted by Czarina Evangelista, Dr. Wayne G. Brake, and Dr. Peter Shizgal.

Chapter 4: These experiments were conceived and designed by Czarina Evangelista, Arne Hantson, Dr. Peter Shizgal, and Dr. Wayne G. Brake. Czarina Evangelista conducted the experiments and analyzed the data. Arne Hantson, Dr. Waqqas Shams, and Dr. Anne Almey assisted with the experimental design and data analysis in experiment 1. Yaman Al-qadri, Brunella V. Gonzalez Cautela, Fei Xiang Zhou, Jesse Duchemin, Andrew Habrich, Victoria Lorenc, Collin Gagne, and Khaoula El Oufi assisted with behavioral training and testing in experiment 1. Michael Pillegi, Jacques, R. Voisard, Vanessa Boulos, Noemie Tito, Ramela Arax Koumrouyan, and Smita Patel helped with behavioral training and testing in experiment 2.

Table of Contents

List of Figures

Chapter 2

Chapter 3

Chapter 4

Chapter 5

List of Tables

Chapter 2

Chapter 3

List of Abbreviations

μA ..... microampere
6-OHDA 6-hydroxydopamine
BLA ..... basolateral amygdala
BSR ..... brain stimulation reward
CI ..... confidence interval
eICSS ..... electrical intracranial self-stimulation
oICSS ..... optical intracranial self-stimulation
D1R ..... dopamine D1 receptor family
D2R ..... dopamine D2 receptor family
FR ..... fixed ratio
HP ..... high priming
Hz ..... hertz
i.p. ..... intraperitoneal
ITI ..... intertrial interval
LH ..... lateral hypothalamus
LP ..... low priming
MFB ..... medial forebrain bundle
ms ..... millisecond
NAc ..... nucleus accumbens
NP ..... no priming
pps ..... pulses per second
PVT ..... paraventricular thalamus
RI ..... random interval
s ..... seconds
s.c. ..... subcutaneous
TDRL ..... temporal-difference reinforcement learning
VI ..... variable interval
VTA ..... ventral tegmental area

Chapter 1: General Introduction

Elucidating the neurobiological basis of reward pursuit holds important implications for understanding both adaptive behaviors such as foraging and pathological behaviors such as binge eating and drug abuse. Decades of research on reward and motivation has implicated dopamine transmission. Advances in that research may have obscured the contributions of non-dopamine neurotransmitter systems. Research on the priming effect of rewards provides evidence that certain aspects of motivation may not depend on dopamine signaling.

1. Terminology

As mentioned by Cofer (1972), many of the terms used in psychology, like *reward* and *motivation,* derive from popular vocabulary. Over the course of borrowing those terms, researchers have given them more specific meanings that are often distant from their popular, non-scientific origins. As such, it is important to define how the terms reward and motivation are used in a scientific setting, specifically in this thesis.

A reward is any goal object (e.g., food, drugs, brain stimulation) that elicits approach and consumption (Young, 1959; White, 1989; Schultz, 2015). On a scorching hot summer day, people may approach trees that offer shade or shops that sell cold, refreshing beverages. In those cases, the shade and cold beverages elicit approach and are thus rewarding.

Two key properties of a boost in motivation are that 1) behavior is *directed* to achieve a specific goal and 2) that goal-directed behavior is *invigorated* (Cofer, 1972). In a T-maze, Deustch *et al.* (1964) showed that thirsty rats typically choose the arm baited with water instead of the opposite arm baited with rewarding brain stimulation. However, when thirsty rats receive a pre-trial sample (i.e., prime) of brain stimulation, they are more likely to choose brain stimulation over water. Thus, their behavior is *directed* toward the primed reward over the competing reward. In another study, Gallistel *et al*. (1974) showed that rats primed with brain stimulation run faster to the end of a runway to earn more rewarding brain stimulation. Thus, goal-directed behavior is *invigorated* following receipt of a prime.

Dopamine transmission encompasses a) the release of dopamine from the presynaptic neuron into the synaptic cleft, b) dopamine binding to receptors located on the postsynaptic neuron and autoreceptors on the presynaptic neuron, and c) receptor-activated second-messenger cascades influencing firing of the postsynaptic neuron. Dopamine transmission has been highly implicated in reward and motivation: Manipulation of dopamine transmission alters goal-directed

behavior (Wise *et al.*, 1978; Randall *et al.*, 2015) and changes in the activity of dopamine neurons accompany different stages of the reward pursuit and procurement (e.g., Mohebi *et al.*, 2019).

Dopamine is released over different timescales, which are commonly used to distinguish phasic and tonic responses (Schultz, 2016). Phasic dopamine release is a short-lasting response produced by burst firing of the dopamine neuron. Behaviorally salient stimuli typically induce phasic dopamine release. Tonic dopamine release is a continuous response that maintains steady-state concentrations of extracellular dopamine. Tonic dopamine depends on the baseline firing of the dopamine neuron, but other factors can also influence tonic dopamine release (Grace, 1991).

In this paper, we review research that demonstrates the importance of dopamine transmission in reward and motivation. We also discuss a motivational phenomenon called the priming effect of rewards that provides evidence that dopamine signaling may not be essential for certain aspects of motivation. Findings from this research highlight the importance of reconsidering the role of non-dopamine neurotransmitter systems in reward and motivation.

2. Dopamine and Reward

Research on the neurobiology of reward began in earnest following the observation by Olds and Milner (1954) that rats are willing to work for electrical stimulation of certain sites in their brains. The catecholamine hypothesis proposes that neurons that release catecholamines, such as norepinephrine and dopamine, mediate electrical intracranial self-stimulation (eICSS) (Poschel & Ninteman, 1963; Stein, 1968; Crow, 1969, 1970, 1972; Crow *et al.*, 1972; Arbuthnott *et al.*, 1970; German & Bowden, 1974). Many anatomical, pharmacological, and lesion studies demonstrate the importance of these catecholamines in eICSS (for reviews see German & Bowden, 1974; Fibiger, 1978; Wise, 1978). However, greater evidence in support of the role of dopamine in reward rather than that of norepinephrine accumulated over time (for reviews see Wise & Rompre, 1989; Wise, 2008). For example, Corbett and Wise (1979) found no correlation between the robustness of eICSS and the density of noradrenergic fibers near the electrode. This led researchers to focus more specifically on dopamine's role in reward.

Currently, reward is most commonly linked with the dopamine system. For example, rewards such as food, drugs, and electrical brain stimulation are associated with increases in midbrain dopamine activity (Fibiger, 1978; Wise, 1978; Wise & Rompre, 1989; Fiorino *et al.*, 1993; You *et al.*, 2001; Phillips *et al.*, 2003; Roitman *et al.*, 2004; Rodeberg *et al.*, 2016). One

particular dopamine projection that has received a lot of attention for its involvement in reward is the mesocorticolimbic dopamine pathway (You *et al.*, 2001; Witten *et al.*, 2011; Steinberg *et al.*, 2014). Those dopamine neurons project from the midbrain (i.e., the ventral tegmental area; VTA) to cortical (e.g., prefrontal cortex; PFC) and limbic structures (e.g., the nucleus accumbens; NAc).

The mesocorticolimbic pathway is one of many circuits that runs through the medial forebrain bundle (MFB) (Nieuwenhuys *et al.*, 1982; Geeraedts *et al.*, 1990a, 1990b). Stimulating various regions of the MFB promotes eICSS (Olds & Milner, 1954; Olds, 1956; Olds & Olds, 1963; Koob *et al.*, 1978; Phillips, 1984). However, the MFB fibers directly responsible for eICSS appear to not be dopaminergic (Yeomans, 1979; Shizgal *et al.*, 1980; Bielajew *et al.*, 1982; Bielajew & Shizgal, 1982; Murray & Shizgal, 1994, 1996a, 1996b). The directly-stimulated MFB fibers are proposed to transsynaptically activate midbrain dopamine neurons to support eICSS (Shizgal *et al.*, 1980; Wise, 1980; Gallistel *et al.*, 1981; Shizgal, 1989; Yeomans, 1989).

Enhancing or inhibiting midbrain dopamine activity increases or attenuates eICSS, respectively (Crow, 1970; Franklin & McCoy, 1979). One interpretation of these findings is that dopamine transmission mediates the rewarding properties of electrical brain stimulation. An alternative interpretation is that manipulating dopamine transmission affects performance capacity (Fibiger *et al.*, 1976). Studies have shown that manipulating dopamine transmission affects reward above and beyond any changes in the capacity to respond (Edmonds & Gallistel, 1977; Franklin, 1978; Franklin & McCoy, 1979; Gallistel & Karras, 1984). For example, Franklin and McCoy (1979) showed that following pimozide administration, a D2 family receptor (D2R) antagonist, rats' responding declines and eventually disappears. Presentation of a Pavlovian cue paired with reward re-establishes responding for brain stimulation. This indicates that pimozide did not abolish performance capacity but instead diminished the rewarding effect of brain stimulation.

Wise proposed the anhedonia hypothesis as a corollary to the dopamine hypothesis of reward (Wise, 1982). This corollary posits that mesocorticolimbic dopamine transmission is involved in the subjective pleasure associated with rewards. Wise *et al.*'s (1978) seminal study on dopamine receptor antagonism and food reward quality was fundamental to the anhedonia hypothesis. D2R blockade with pimozide attenuates lever pressing for food, but only if the food

was previously experienced with pimozide. It was thought that disrupting dopamine transmission removes the pleasurable properties of the food and thus causes the decline in lever pressing for food across tests.

Wise (2008) later clarified that the anhedonia hypothesis proposes only a partial relationship between dopamine transmission and pleasure since rewards are often—but not always—associated with pleasure. Lamb *et al.* (1991) observed that participants are willing to work for low doses of heroin despite not reporting pleasurable effects. Correlations between dopamine transmission and the pleasurable effects of drugs of abuse are weak (Volkow *et al.*, 1999; Drevets *et al.*, 2001). Additionally, rodent studies have shown that implicit facial reactions, which are interpreted to reflect the hedonic value of reward, to palatable rewards persist following dopamine receptor antagonism (Peciña *et al.*, 1997). Regardless of dopamine's role in pleasure, it is evident that dopamine transmission is important for reward.

2.1 Summary of the Role of Dopamine in Reward

Decades of research has implicated dopamine transmission in reward. There is evidence that perturbations of dopamine transmission affect reward seeking independent of dopamine's effect on performance. The anhedonia hypothesis posits that dopamine transmission also mediates pleasure experienced from rewards, but the evidence is equivocal. Although the specific contributions of dopamine transmission in reward are yet to be fully understood, it is clear that dopamine transmission plays an integral role in reward pursuit.

3. Dopamine and Motivation

Motivated behavior is influenced by many internal and external factors. Hull (1943) proposed that entering a state of deprivation or need (e.g., hunger or thirst) disrupts the body's equilibrium and activates a motivational state called *drive*. In what he referred to as the drive-reduction theory of motivation, it was proposed that *drive* energizes behavioral responses to reduce the state of need and restore homeostasis. According to this idea, rewards serve to reduce the state of need. For example, rats consume food to alleviate hunger or drink water to alleviate thirst. It has been shown that hungry or thirsty rats are quicker to start consuming food or water, respectively, than when they are sated (Kimble, 1951; Bolles, 1962). Food or water deprivation also causes rats to lever press more for food or water, respectively (Collier & Levitsky, 1967). These studies support the notion that motivated behavior is a response to need.

However, the drive-reduction theory does not explain all motivated behavior. Humans and rats alike may eat food even when they are not hungry. We may eat to avoid future hunger (Collier *et al.*, 1977), or because we eat on a regular schedule (Siegal, 1961) or because the food itself is desirable (Barbano & Cador, 2005; Lowe & Butryn, 2007). Additionally, rats work for MFB stimulation, which induces feeding (Hoebel & Teitelbaum, 1962; Margules & Olds, 1962). In other words, MFB stimulation induces drive rather than attenuating it. Consistent with that phenomenon is the idea of *incentive motivation*, which holds that rewards and reward-related cues can augment motivation and thereby influence reward pursuit (Bindra, 1969, 1978; Bolles & Moot, 1973; Toates, 1981, 1986).

3.1 Incentive Salience

Robinson and Berridge (1993) proposed that the psychological experiences of *wanting* and *liking* are involved in incentive motivation, and separate neural circuits are thought to mediate those two processes. The midbrain dopamine system is thought to be the neural substrate of incentive salience, which is what makes rewards and reward-related cues attractive and wanted. Thus, the dopamine system is hypothesized to directly play a role in wanting but not liking (Robinson & Berridge, 1993; Berridge & Robinson, 1998).

Changes in wanting or liking are interpreted by certain behavioral measures. Vigor of goal-directed behavior is one measure thought to reflect the incentive salience of reward. Wise *et al.* (1978) noted a similar decline in responding for food when reward was omitted and when responding was rewarded but D2Rs were blocked. One interpretation of these results is that the incentive salience of reward relies on D2R signaling.

The contributions of dopamine transmission in wanting have also been well documented in studies that use a reinstatement model of drug relapse (de Wit & Stewart, 1981, 1983; Self & Nestler, 1998; Shaham *et al.*, 2003). In this model, rats are trained to self-administer drugs of abuse such as cocaine or heroin (de Wit & Stewart, 1981; 1983). Later, they are forced into a period of abstinence when operant responding no longer delivers the drug, resulting in extinction of the drug-seeking behavior. A subsequent presentation of a non-contingent sample of the drug (priming) reinstates drug seeking. It is proposed that the drug prime promotes wanting by enhancing the incentive salience of the drug and drug-associated cues (Leri & Stewart, 2001; Robinson *et al.*, 2013).

Drugs of abuse are not the only substances that can re-direct and re-invigorate goal-seeking behavior in a reinstatement paradigm. Priming with the D2R agonist, bromocriptine, reinstates cocaine seeking (Wise *et al.*, 1990). In contrast, blocking D2Rs with haloperidol prevents amphetamine-induced reinstatement (Ettenberg, 1990). These results demonstrate the importance of D2R signaling in wanting.

Liking is commonly inferred from reward seeking because it is thought that rewards that are wanted are also liked. This assumption may be incorrect considering that addicts continue to seek drugs although they reportedly no longer experience pleasure from those drugs (Lamb *et al.*, 1991; Robinson & Berridge, 1993; Robinson *et al.*, 2013). One behavioral measure thought to reflect changes in liking is the taste reactivity test (for reviews see Berridge, 2000; Steiner *et al.*, 2001). Hedonic taste reactions can be expressed as rhythmic tongue protrusions and aversive responses can be expressed as gaping. Rats maintain hedonic responses to sucrose following dopamine receptor antagonism (Peciña *et al.*, 1997) or lesions of the mesolimbic dopamine system (Berridge & Robinson, 1998). Additionally, microinjections of amphetamine into the NAc shell potentiates cue-induced instrumental responding for sucrose but not hedonic reactions to sucrose (Wyvell & Berridge, 2000). These findings suggest that dopamine transmission is involved in wanting but not liking of rewards.

3.2 Strength and Cost of Reward

Other variables that affect motivation to pursue rewards are the *strength* and *cost* of reward. Previous studies showed that cocaine enhances eICSS by lowering the strength of brain stimulation required to support eICSS (Esposito *et al.*, 1978; Bauco & Wise, 1997). By elevating synaptic dopamine through inhibition of dopamine reuptake, cocaine was thought to increase the sensitivity of neural circuitry involved in reward (i.e., brain reward circuitry) and thus lower the strength of brain stimulation necessary to sustain eICSS. Based on this, dopamine transmission affects reward intensity. Another interpretation is that dopamine transmission alters the subjective cost of reward (Salamone *et al.*, 2007, 2009; Hernandez *et al.*, 2010; Trujillo-Pisanty *et al.*, 2014). For example, elevated dopamine levels may enhance eICSS by increasing willingness to exert effort or attenuating the perceived effort. Shizgal and colleagues (Arvanitogiannis & Shizgal, 2008; Hernandez *et al.*, 2010) proposed that the conventional methods used to measure reward seeking are unable to fully distinguish dopamine's role in reward strength versus reward cost.

Historically, two methods were commonly used to investigate reward seeking, particularly for brain stimulation. In the first method, response rates were observed only at a single value of reward strength (e.g., a single pulse frequency). The effect of cocaine was examined by measuring how it changes response rates for brain stimulation at a single value of reward strength. Cocaine may have potentiated responding for brain stimulation by increasing the sensitivity of brain reward circuitry (Crow, 1970) or decreasing the subjective reward cost (Salamone *et al.*, 1997, 2003, 2005; Hernandez *et al.*, 2010; Trujillo-Pisanty *et al.*, 2014). There is no way to distinguish between these two interpretations with this method.

It was thought that a curve-shift method could separate the effects of reward strength or cost (Edmonds & Gallistel, 1974; Miliaressis *et al.*, 1986). With this method performance is measured at varying reward strengths as opposed to a single value of reward strength, and response rate is often used as the dependent variable. Reward seeking is highest when brain stimulation is intense and decreases in an S-shaped fashion as the stimulation weakens. In such cases, the sigmoidal function mapping stimulation strength into performance is called a "rate-frequency" function.

Interactions between drugs and electrical brain stimulation are indexed by lateral or vertical shifts of the curve. Lateral shifts were interpreted to reflect to changes in reward-system sensitivity and vertical shifts were thought to reflect changes in subjective reward cost (Edmonds & Gallistel, 1974). However, it was later shown that changes in both reward strength and cost produce similar lateral shifts in the curve (Frank & Williams, 1985; Fouriezos *et al.*, 1990). These findings demonstrate that it was incorrect to assume that lateral shifts exclusively reflect changes in reward-system sensitivity. Thus, a curve-shift method does not reveal the degree to which a change in reward pursuit is due to a change in reward-system sensitivity or subjective cost.

To reduce the ambiguity of which stages of processing neurochemical manipulations change in reward pursuit, Arvanitogiannis and Shizgal (2008) developed the reward-mountain model, which measures reward seeking as a function of both the strength and cost of reward. This produces a three-dimensional structure, referred to as a reward mountain, that has two horizontal axes that represent reward strength and reward cost, and a vertical axis that represents performance. The net contribution of reward intensity and subjective costs is called the payoff from brain stimulation. To determine behavioral allocation toward working for brain stimulation,

the payoff from brain stimulation is compared to the payoff from alternative activities such as grooming and resting. Reward seeking is highest when the payoff is high (e.g., when the reward is intense and cost is low) and declines in an S-shaped fashion as the payoff decreases (e.g., when the reward is weak and cost is high).

In an extension of the curve-shift method, interactions between drugs and brain stimulation are indexed by shifts in the reward mountain (Arvanitogiannis & Shizgal, 2008). A vertical shift in the reward mountain indicates a change in reward-system gain. This rescales the reward-intensity function to produce equal, proportional changes in the intensity of all reward values. Thus, altering reward-system gain changes the absolute value of rewards. A shift along the reward-strength axis indicates a change in reward-system sensitivity. This changes the stimulation strength required to produce a given reward intensity, but it does not change the maximum intensity. Thus, altering reward-system sensitivity changes the relative value of rewards. A shift along the reward-cost axis indicates a change in the value of competing activities, reward-system gain, and/or subjective costs. Two of such subjective costs include 1) opportunity cost, which is the work time required to earn a reward, and 2) effort cost, which is the physical effort exertion required to obtain a reward. The reward-mountain model cannot distinguish changes among the value of competing activities, reward-system gain, or subjective costs.

It had been proposed that changes in dopamine transmission affect eICSS by altering reward-system sensitivity (Crow, 1970; Esposito *et al.*, 1978). The application of the reward-mountain model shows that this is not the case. Rats treated with a dopamine reuptake inhibitor, cocaine or GBR-12909, work for rewarding brain stimulation at higher costs (Hernandez *et al.*, 2010, 2012). Boosting dopamine tone may have decreased subjective costs, increased reward-system gain, and/or decreased the value of competing activities. D2R antagonism with pimozide diminishes willingness to work for rewarding brain stimulation at high costs (Trujillo-Pisanty *et al.*, 2014). Thus, blocking D2Rs may have increased subjective costs, decreased reward-system gain, and/or increased the value of competing activities. These findings indicate dopamine tone influences reward pursuit by affecting subjective costs, reward-system gain, and/or the value of competing activities rather than by affecting reward-system sensitivity.

3.3 Effort Cost

Midbrain dopamine activity, specifically those dopamine neurons projecting to the NAc, is proposed to regulate the effort cost of reward (Salamone *et al.*, 2003, 2005, 2007, 2009; Salamone & Correa, 2012). Salamone and colleagues have investigated this theory by using ratio schedules to manipulate effort cost and by impairing NAc dopamine transmission. Aberman and Salamone (1999) trained rats to press a lever for a fixed number of times (FR schedule), ranging from one to 64 presses, to earn a palatable food reward. NAc dopamine was depleted with 6-hydroxydopamine (6-OHDA). At FR64, responding of non-treated rats was greater by ten-fold than dopamine-depleted rats. Similar results were observed when the ratio requirement ranged from FR5 to FR300 (Salamone *et al.*, 2001). Dopamine-depleted rats are not willing to work as hard as non-treated rats when the effort cost is high.

Manipulating effort via ratio requirement also affects the time requirement to obtain rewards. Higher FR schedules require more time to complete compared to lower FR schedules. To dissociate the effect of dopamine depletion on time and ratio requirements, Salamone and colleagues used variable interval (VI) and FR schedules in tandem. On a VI schedule, an average variable period of time must elapse before a response is rewarded. For example, on a 30-second (s) VI (VI30) schedule, a response is rewarded when it is made after 30 s have elapsed, on average. In a tandem VI-FR schedule, the interval requirement is followed by a ratio requirement. On a VI30-FR5 schedule, in addition to waiting for 30 s, on average, the rat is required to press five times to earn a reward.

Dopamine depletion does not alter responding on a VI30 schedule but responding is attenuated on a tandem VI30-FR5 schedule (Correa *et al.*, 2002). In a wide variety of VI and tandem VI-FR schedules, response rates are reduced in dopamine-depleted rats only in the tandem VI-FR schedule (Mingote *et al.*, 2005). These findings are consistent with the idea that NAc dopamine affects the effort cost of reward. However, dopamine depletion may produce a similar decline in responding when reward strength is systematically manipulated. Thus, it is unclear whether the findings by Salamone and colleagues are due to a change in reward intensity or subjective effort cost.

3.4 Response Vigor

Although there is substantial empirical evidence highlighting the importance of dopamine transmission in vigor (Beierholm *et al.*, 2013; Hamid *et al.*, 2015; du Hoffmann & Nicola, 2016;

Mohebi *et al.*, 2019), there has been a lack of computational models that attempt to explain dopamine's role. Theories on incentive salience (Robinson & Berridge, 1993; Berridge & Robinson, 1998) and effort cost (Salamone & Correa, 2002) do not discuss computational explanations for dopamine's role in vigor. The reward-mountain model (Arvanitogiannis & Shizgal, 2008; Hernandez *et al.*, 2010) offers a computational model but it is not designed to directly study response vigor. Reinforcement learning models provide potential explanations on how animals learn to select actions but they do not explain how much vigor an animal will exert on those actions (Montague *et al.*, 1996; Schultz *et al.*, 1997; for review see Colombo, 2014).

Niv *et al.* (2007) developed the model of optimal responding to address vigor and the role of dopamine transmission. In this model, vigor is determined by weighing the cost of behaving quickly against the cost of delaying future rewards (Niv, 2007; Niv *et al.*, 2007). It is assumed that behaving quickly is effortful because otherwise responding should always be performed vigorously (Niv, 2007). The cost of delaying future rewards reflects the maximum reward rate minus the cost. Other descriptions of the cost of delaying future rewards are the cost of wasting time or the net reward rate. Imagine that the net reward rate is one reward per second. Every second the operant response is performed leads to an average reward gain. Correspondingly, every second the operant response is not performed leads to an average reward loss (i.e., cost of wasted time). This could explain why hungry rats perform all actions at faster rates (Hull, 1943; Niv *et al.*, 2005, 2006) A high net reward rate puts greater pressure to generally respond at a faster rate to maximize the acquisition of rewards and minimize reward loss (Niv *et al.*, 2007).

Vigor is proposed to be signaled by tonic firing (Niv *et al.*, 2006, 2007; Niv, 2007). Since motivational states such as hunger have general effects on response vigor for all actions, it was thought that the signal that influences vigor should reflect this generalized effect. Niv hypothesizes that tonic dopamine in the basal ganglia and some prefrontal areas signal the cost of wasted time. More specifically higher concentrations of tonic dopamine are proposed to increase the cost of wasted time to potentiate vigor. Conversely, lower tonic dopamine levels decrease the cost of wasted time, resulting in attenuated vigor. To test these hypotheses, Niv *et al.* (2006) decreased the cost of wasted time by 60% in their computational model. This lowers the rate of responding, which is similar to the effect of dopamine depletion observed by Salamone and colleagues (Aberman & Salamone, 1999; Mingote *et al.*, 2005). This evidence supports the hypothesis that tonic dopamine affects vigor.

3.5 Summary of the Role of Dopamine in Motivation

Two key features of an enhancement in motivation are that 1) behavior is directed toward a specific goal and 2) that goal-directed behavior is invigorated. The incentive salience hypothesis proposes that dopamine transmission controls motivation by mediating the incentive salience, or attractiveness, of reward but not the pleasurable aspects of it. An alternative idea is that dopamine transmission mediates the cost of reward. Results from the reward-mountain model are consistent with the idea that subjective costs are modulated by tonic dopamine. There is also evidence that dopamine transmission affects the effort cost of reward. The model of optimal responding proposes that tonic dopamine determines the vigor of actions. The variation in these theories emphasizes that the mechanisms of dopamine transmission in motivation are complex and still to be determined.

4. The Priming Effect of Rewards

Receipt of reward boosts motivation to work for more, which is a phenomenon called *the priming effect of rewards* (Gallistel, 1966; Reid *et al.*, 1973; Gallistel *et al.*, 1974; Stellar & Gallistel, 1975). For example, the taste of one chip may lead to wanting another chip. This is different from the identically named priming effect observed in a reinstatement model of drug relapse (de Wit & Stewart, 1981, 1983). In the latter, drug seeking is learned and then extinguished. Non-contingent delivery of a reward (priming) can re-establish the previously extinguished drug-seeking behavior. That priming effect is also commonly referred to as priming-induced reinstatement. In contrast, the priming effect of rewards invigorates a well-established behavior that has not undergone extinction.

4.1 The Priming Effect of Electrical Brain Stimulation

The majority of the research on the priming effect of rewards have been conducted using electrical brain stimulation as the reward and have used a runway paradigm (Gallistel, 1966, 1969a, 1969b; Reid *et al.*, 1973; Edmonds & Gallistel, 1974; Gallistel *et al.*, 1974; Stellar & Gallistel, 1975; Wasserman *et al.*, 1982). The runway generally consists of a start box, an alley, and a goal box. Rats are primed with non-contingent brain stimulation in a start box. Following a delay, a start door opens to give the rats access to the alley. Rats travel to a goal box located at the end of the alley, which contains a lever that delivers rewarding brain stimulation when pressed.

Two distinct effects of electrical brain stimulation have been measured in the runway paradigm: a priming effect and a rewarding effect. The priming effect of the non-contingent brain stimulation received in the start box is expressed as a transient increase running speed to travel to the goal box to lever press for brain stimulation. The rewarding effect of the response-contingent brain stimulation received in the goal box is expressed as the proclivity of the rat to run down the alley and the value it assigns to the stimulation available there. Gallistel *et al.* (1974) showed that a change in the strength of response-contingent goal-box stimulation leads to gradual adjustments of performance over multiple trials. Once the rat learns the updated value of the stimulation, a new, stable performance level is attained. In contrast, they also demonstrated that performance adjusts immediately following a change in the strength of the non-contingent start-box stimulation. Moreover, they showed that the effect of priming stimulation is a systematic function of response-contingent goal-box stimulation. If the rat learns that the rewarding goal-box stimulation is weak and without value, no amount of priming would induce the rat to run down the alley. These results indicate that the priming effect and rewarding effect of brain stimulation are independent.

The priming effect of rewards manifests two defining properties of an increase in motivation: it *directs* and *invigorates* reward-seeking behavior (Cofer, 1972). In a T-maze, Deutsch *et al.* (1964) offered thirsty rats a choice between water and brain stimulation. Rats primed with brain stimulation are more likely to choose the arm that delivers brain stimulation. Thus, priming *directs* behavior toward pursuing a primed reward over a competing reward. In a runway paradigm, rats run faster to the goal box after having received priming stimulation in the start box (Gallistel, 1966; Reid *et al.*, 1973; Gallistel *et al.*, 1974; Stellar & Gallistel, 1975). Thus, priming *invigorates* reward seeking.

4.2 The Priming Effect of Food and Drugs

Although the priming effect of rewards has mainly been studied with electrical brain stimulation as the reward, there is also evidence that it extends to other rewards such as food and drugs (for review see de Wit, 1996). Previous research on the priming effect of food used mazes or runways. van der Kooy and Hogan (1978) used a square maze with each corner baited with food pellets. There was a delay before each opportunity to run to the next corner (ITI). The ITIs varied from short to long intervals. In other words, the reward received in each corner also

served as a prime. It was shown that hamsters run faster to the next corner following shorter ITIs (10 s), which constitutes as a priming effect.

Terry (1980, 1983) studied the priming effect of food using a runway paradigm comparable to the one used by Gallistel and colleagues (Edmonds & Gallistel, 1974; Gallistel *et al.*, 1974; Stellar & Gallistel, 1975). A trial starts after a 0.5-minute or 5-minute delay from consuming all the priming food pellets. A priming effect is seen at the 0.5-minute delay on the first day but then largely disappears with further training. Another study showed that the detection of a priming effect depends on whether comparisons were made within- or between-subjects (Terry, 1983). Thus, unlike with brain stimulation, there is more variability in the incidence of a priming effect of food.

The priming effect associated with drugs is usually priming-induced reinstatement. That involves extinction of a learned behavior; thus, it is different from the priming effect of rewards. However, studies on the priming effect of drugs conducted in humans rarely involve extinction. Participants may abstain from using drugs, but this is not equivalent to extinction. Because of this, the priming effect of drugs studied in humans could be considered comparable to the priming effect of rewards discussed in this paper.

Most studies on the priming effect of drugs have concerned alcohol. Alcohol-dependent participants report increased craving for alcohol in response to priming (Ludwig & Wikler, 1974). Bigelow *et al.* (1977) showed that priming alcohol-dependent participant increases their willingness to work for alcohol. Stockwell *et al.* (1982) showed that primed alcohol-dependent participants consume alcohol faster. These studies demonstrate that alcohol-dependent participants are sensitive to the priming effect of alcohol.

The priming effect of alcohol has also been observed in social drinkers. In an experiment conducted by Chutuape *et al.* (1994), social drinkers performed two separate tasks: one task earned them money and another earned them alcohol. The probability of winning money varied from low to high, and the probability of winning alcohol was constantly moderate. Following priming, social drinkers work more for alcoholic beverages when the probability of earning money is low and they report greater desire for alcohol. Interestingly, Kirk and de Wit (2000) used the same methods as Chutuape *et al.* (1994) but they did not find that alcohol priming increased the probability of choosing alcohol over money in the choice task. Although social drinkers are also susceptible to the priming effect of alcohol, there is variability in its incidence.

Other drugs, such as cocaine, can also induce a priming effect. Jaffe *et al.* (1989) showed that cocaine users report higher craving and wanting for cocaine in response to priming with cocaine or the D2R agonist bromocriptine. Priming-induced craving and wanting diminish over time, which demonstrates the transient nature of the priming effect (Deutsch *et al.*, 1964; Gallistel, 1966).

The priming effect of rewards does not exclusively apply to electrical brain stimulation. Food priming produces a short-lasting invigoration of food seeking. Accordingly, drug priming directs and invigorates drug seeking. Compared to electrical brain stimulation, there is greater variability in the incidence of a priming effect of food or drugs.

4.3 Dopamine and the Priming Effect of Electrical Brain Stimulation

There is abundant evidence that dopamine transmission is important for reward (Edmonds & Gallistel, 1977; Franklin, 1978; Franklin & McCoy, 1979; Gallistel & Karras, 1984) and motivation (Robinson & Berridge, 1993; Berridge & Robinson, 1998; Niv *et al.*, 2005, 2007; Niv, 2007; Hernandez *et al.*, 2010, 2012; Salamone & Correa, 2012; Trujillo-Pisanty *et al.*, 2014; Salamone *et al.*, 2016). Prior to this thesis, there has only been one study that investigates the role of dopamine transmission in the priming effect.

Wasserman *et al*. (1982) administered pimozide, a D2R antagonist, and then examined whether this blocks the priming effect. Following high doses of pimozide, thirsty rats primed with electrical brain stimulation continue to choose brain stimulation more often than water. Additionally, primed rats continue to run faster to the goal box to earn rewarding brain stimulation. They show a priming effect in the first few trials and then cease to perform altogether. Since the rewarding effect of brain stimulation is sensitive to dopamine receptor antagonism (Gallistel *et al*., 1982), administration of pimozide diminishes performance across the session. Nevertheless, a priming effect of rewards is present prior to pimozide blocking the rewarding effect of brain stimulation. Therefore, D2R antagonism with pimozide fails to eliminate the goal-directing and energizing effects of priming.

4.4 Summary of the Priming Effect of Rewards

Receipt of a reward enhances motivation to work for more, which is called the priming effect of rewards. This phenomenon occurs with electrical brain stimulation, food, and drugs of abuse. Based on dopamine's role in incentive motivation, dopamine transmission would be

expected to mediate the priming effect. On the contrary, there is evidence that indicates that the priming effect may not depend on dopamine signaling.

5. Rationale and Hypotheses

The majority of the research on reward and motivation has focused on the contributions of the dopamine system. As a consequence, the complementary roles of other non-dopamine systems may have been insufficiently studied. There is evidence from previous research that certain motivational phenomenon, such as the priming effect of electrical brain stimulation, may not depend on dopamine transmission.

The goal of the present thesis was to investigate the priming effect of rewards and the role of dopamine transmission. To study this, we developed a new paradigm to measure the priming effect, we examined variables that affect priming, and we assessed whether priming generalizes to other rewards. Lastly, we investigated whether the priming effect depends on dopamine receptor signaling.

In Chapter 2, we developed a new method to measure the priming effect of electrical brain stimulation using a standard operant-conditioning paradigm that indexes changes in motivation based on lever-pressing behavior. Chapter 2 also investigated whether the priming effect of electrical brain stimulation is affected by reward strength, reward cost, or both. We hypothesized that this novel method can measure a priming effect of electrical brain stimulation and that the incidence of a priming effect depends on the strength and cost of reward.

In Chapter 3, we aimed to make our design more analogous to a runway after observing high variability in the incidence of a priming effect in Chapter 2. Additionally, we hoped to reduce the potentially aversive effect of the high-frequency, free priming stimulation by allowing rats to self-administer the priming stimulations. We posited that these methodological changes would produce a more consistent priming effect comparable to those observed by Gallistel and colleagues (Reid *et al.*, 1973; Gallistel *et al.*, 1974).

Most of the previous research on the priming effect used brain stimulation as a reward and the limited research on the priming effect of food were inconsistent. In Chapter 3, we examined whether the priming effect generalizes to other rewards such as food. We predicted that with our design we would show that food elicits a priming effect.

Lastly, Chapters 3 and 4 tested whether the priming effect of electrical brain stimulation or food, respectively, is eliminated by selective dopamine receptor antagonists. Wasserman *et al.*

(1982) previously tested the priming effect against pimozide, which binds to D2Rs, D3Rs, 5-HT_7 receptors. It also has very low affinity for D1Rs. Prior to this thesis, the priming effect of food has not been challenged with dopamine receptor antagonists. We employed more selective dopamine receptor antagonists to better discern the role of dopamine transmission in the priming effect. We hypothesized that the priming effect persists following dopamine receptor blockade.

Chapter 2: The Priming Effect of Electrical Brain Stimulation Depends on the Strength and Cost of Reward

Abstract

Receipt of an intense reward boosts motivation to work for more of that reward. This phenomenon is called the *priming effect of rewards*. Since motivation to pursue a reward is affected by variables such as the strength and cost of reward, the priming effect might also depend on these variables. A new method was developed for measuring the priming effect of electrical brain stimulation that relies on lever-pressing behavior. Using this method, performance was measured as a function of reward strength or cost. We observed how those variables impact the priming effect. Lastly, we examined whether the priming effect is eliminated with the administration of a dopamine D2 receptor family antagonist. Our findings indicate that the priming effect of electrical brain stimulation is sensitive to reward strength and cost. Additionally, unlike other motivational constructs, the priming effect may not depend on dopamine signaling.

1. Introduction

Receipt of an intense reward boosts motivation to seek a subsequent reward. This phenomenon is called the *priming effect of rewards*. It is different from the priming effect observed in a reinstatement model of drug relapse (de Wit & Stewart, 1981, 1983). In that model, drug seeking is learned and then extinguished. Non-contingent delivery of a reward (priming) can re-establish the previously extinguished drug-seeking behavior. That phenomenon is also commonly referred to as priming-induced reinstatement. In contrast, the priming effect of rewards discussed here invigorates a well-established behavior that has not undergone extinction.

Most studies that examined the priming effect of rewards have been conducted in rats working for electrical brain stimulation in a runway paradigm (Reid *et al.*, 1973; Edmonds & Gallistel, 1974; Gallistel *et al.*, 1974; Stellar & Gallistel, 1975). The runway apparatus consists of a start box, an alley, and a goal box. Before a trial starts, rats are placed in the start box and access to the alley is blocked by a start door. A trial starts when the start door opens, which permits access to the alley. Rats travel to the goal box located at the end of the alley, which contains a lever that delivers rewarding brain stimulation when pressed. Receipt of priming stimulation in the start box potentiates running to the goal box to lever press for rewarding brain stimulation.

In that runway paradigm, running speed is measured as an index for change in motivation. Faster running speeds indicate a boost in motivation. However, this measure presents an issue because brain stimulation produces locomotor movement of the hind legs that can potentiate running speed. Electrical stimulation of midbrain regions such as the lateral hypothalamus (LH) has been shown to cause stepping movements of the hind legs in awake (Sinnamon & Sklow, 1990) and anesthetized (Sinnamon *et al.*, 1987; Levy & Sinnamon, 1990) rats. Thus, the increase in running speed thought to be induced by priming stimulation could be contaminated by the hind-limb locomotor effect of brain stimulation. Based on this, the effect of priming stimulation on motivation should be measured independent of the hind-limb locomotor effect of brain stimulation.

The priming effect of electrical brain stimulation manifests two key characteristics of a boost in motivation: it *directs* and *energizes* goal-seeking behavior. Rats have been shown to direct their goal-seeking behavior toward the primed reward over competing rewards (Deutsch *et al.*, 1964). Additionally, that goal-directed behavior is energized when rats are primed (Gallistel

et al., 1974). Tasks designed to measure the priming effect should take into consideration variables that influence motivation to pursue rewards, such as reward strength and cost.

Reward seeking is highest when brain stimulation is intense and decreases in an S-shaped fashion as the stimulation weakens (Edmonds & Gallistel, 1974; Miliaressis *et al.*, 1986). Pulse frequency is typically used as the strength variable in electrical intracranial self-stimulation (eICSS) studies, and response rate is often used as the dependent variable. In such cases, the sigmoidal function mapping stimulation strength into response rate is called a "rate-frequency" function. Priming stimulation stretches rate-frequency curves vertically (Edmonds & Gallistel, 1974). In other words, it enhances performance substantially when the reward is intense, moderately when the reward is intermediate, and trivially when the reward is weak.

Reward seeking is also affected by reward cost. These are of at least two types: opportunity and effort costs. Opportunity cost is the work time required to obtain a reward. Effort cost is the physical effort exertion required to earn a reward. Reward seeking is highest when the opportunity cost is low and decreases as the cost grows (Hernandez *et al.*, 2010, 2012; Trujillo-Pisanty *et al.*, 2014). Similarly, rats are less willing to work as the effort cost increases (Aberman & Salamone, 1999; Salamone *et al.*, 2001). The impact of reward cost on the priming effect has not been documented previously.

The goals of the present study were 1) to design a new task to measure the priming effect of electrical brain stimulation, 2) to examine whether the priming effect depends on reward strength, cost, or both, and 3) to use the new task to study the neurochemical basis of the priming effect. In experiment 1, we assessed a new method for measuring the priming effect of electrical brain stimulation that does not rely on running speed. This was achieved by using standard operant-conditioning chambers and measuring lever-pressing behavior. The locomotor effect of brain stimulation seems to be localized to the hind legs and thus should not contaminate lever-pressing behavior in this paradigm. Experiment 2 observed how reward strength and cost impact the priming effect. Performance was measured as a function of reward strength or cost and it was observed how priming stimulation shifts those functions. In experiment 3, the effects of pimozide, a dopamine D2 receptor family (D2R) antagonist, were examined. Wasserman *et al.* (1982) has provided evidence that the priming effect of electrical brain stimulation may not be mediated by dopamine transmission. We hypothesized that the present method would enable measurement of the priming effect of electrical brain stimulation in a manner uncontaminated by

the hind-limb locomotor effect of brain stimulation. Secondly, we predicted that the priming effect would depend on both the strength and cost of reward. Lastly, we expected that the priming effect of electrical brain stimulation measured in the present behavioral design would persist following D2R antagonism.

2. Method

2.1 Subjects

Male Long-Evans rats (bred at Concordia University, n = 9 in experiment 1, n = 8 in experiment 2 &3) were pair-housed in Plexiglas® cages (46 cm length x 26 cm width x 21 cm height) located in a vivarium with a reversed 12-hour light-dark cycle (lights off from 0800 to 2000 h). Throughout the study, rats had *ad libitum* access to food and water. A mix of Teklad corncob and Sani-Chips® (Envigo, Madison, Wisconsin, USA) were used as bedding and cages were enriched with shredded paper and a tunnel toy. After the rats received bilateral electrode implantations, they were housed individually for the remainder of the experiment. Behavioral tests were conducted during the dark phase of the diurnal cycle. The protocols used were in accordance with guidelines established by Concordia University's Animal Research Ethics Committee's Terms of Reference and the Canadian Council on Animal Care's Guide to the Care and Use of Experimental Animals.

2.2 Electrode Implantation

Each rat weighed at least 350 g at the time of surgery. Ketamine-xylazine (10 mg/kg, Bioniche, Belleville, ON, Canada; Bayer Healthcare, Toronto, ON, Canada) was administered intraperitoneally (i.p) to induce anesthesia. This was followed by a subcutaneous (s.c.) injection of atropine sulfate (0.05 mg/kg, Sandoz, Boucherville, QC, Canada) to reduce bronchial secretions, and penicillin (0.3 ml, s.c., Vetoquinol, Lavaltrie, QC, Canada) to prevent infections. Xylocaine jelly (AstraZeneca, Mississauga, ON, Canada) was applied to the external auditory meatus to diminish discomfort due to the stereotaxic ear bars. After placing the rat in the stereotaxic frame, a mixture of isoflurane and oxygen (Pharmaceutical Partners of Canada Inc., Richmond Hill, ON, Canada) was delivered through a snout mask to maintain anesthesia. Four to six burr holes were drilled into the skull and stainless-steel screws were threaded. The free end of the current-return wire was wrapped around two skull screws (which served as the anode), and the opposite end was terminated in a gold-plated Amphenol connector. Monopolar stainless-steel electrodes were custom-made from insect pins (size: 000) insulated with Formvar enamel,

leaving 0.5 mm of tip bare. The unsharpened end was soldered to a copper wire that was attached to a gold-plated Amphenol connector. Electrodes were bilaterally aimed at the LH level (AP: -2.8 from bregma, ML: ±1.7, DV: -8.8-9.0 from skull surface) of the medial forebrain bundle (MFB) and secured to the skull with dental acrylic. The Amphenol connectors were inserted into a McIntyre miniature connector (Scientific Technology Centre, Carleton University, Ottawa, ON, Canada) that was attached to the skull and skull-screw anchors using dental acrylic. The rats were allowed at least one week to recover from the surgery before self-stimulation training commenced. See Figure 1 for electrode placements.

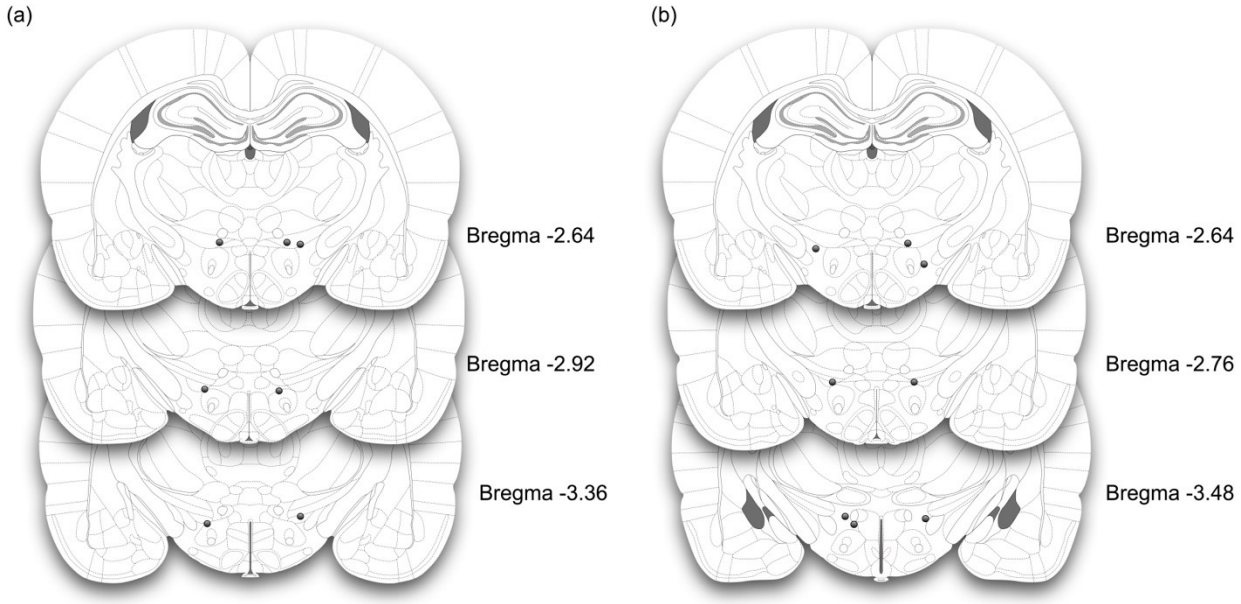

Figure 1. Placement of electrode tips of rats in (a) experiment 1 and (b) experiments 2 and 3. Each electrode tip was located within the boundary of the LH level of the MFB, as determined by the Paxinos and Watson (2007) atlas. Due to issues with tissue collection and histology, electrode placements are missing from two rats (17 and 21) that completed experiment 1.

2.3 Apparatus

The operant chambers (34 cm long x 24 cm wide x 66 cm high) were composed of wire-mesh floors (8 cm above the base), a transparent Plexiglas front panel, an amber house light (10 cm above the mesh floor), and two retractable levers (ENV-112B, MED Associates, St. Albans, Vermont, USA). A lever was located on the left and right sides of the box and a cue light (1 cm) positioned 4 cm above each lever. An electrical swivel centered at the top of the box allowed animals to move freely with the stimulation leads (Figure 2a).

The temporal parameters of the electrical stimulation and pulse amplitude were determined by a computer-controlled digital pulse generator and constant-current amplifier, respectively. Experiments were controlled by, and data were collected with, a custom-written computer program ("PREF3", Steve Cabilio, Concordia University, Montreal, QC, Canada).

2.4 Procedures: Experiment 1

Training. Rats were each screened to determine which electrode (left or right hemisphere) and which electrical current promoted vigorous lever pressing with minimal to no motor effects. The rats in this experiment responded to currents between 264 to 650 microamperes (μA). This is a common range of current that promotes eICSS of the LH (Hernandez *et al.*, 2007, 2012; Solomon *et al.*, 2015). The settings determined for each rat were used throughout the subsequent experiments.

Rats were trained to lever press for electrical brain stimulation on a 10-second (s) variable interval (VI10) schedule. The rewarding stimulation consisted of a single 0.5-s train of 0.1-milisecond (ms) cathodal pulses at 178 pulses per second (pps). Once the operant behavior was stable, rats were trained on the testing procedures. Rats learned to press a setup lever that did not deliver reward but instead activated the extension of a separate reward lever located on the opposite wall of the chamber. The purpose of the setup lever was to position the rat in the same location before each trial and to prevent superstitious behaviors.

Testing. Rats initially underwent a warm-up session, during which they pressed a single lever for electrical brain stimulation. Immediately after this warm-up, testing commenced. As illustrated in Figure 2b, before a trial started there was a 70-s inter-trial interval (ITI), during which the levers were retracted. The first component of the ITI was a waiting period and the house light remained off. The waiting period was followed by 10 s of pretrial cues that signaled the start of a trial. Cues included flashing of the amber house light (10 cycles of 0.5s on, 0.5s off)

and delivery of electrical stimulation on primed trials but not on non-primed trials. The priming stimulation consisted of 10 separate 0.5-s trains of 0.1 ms cathodal pulses delivered at a rate of one train per second. At the end of the pretrial cue period, the house light turned off and there was a five-s delay. At the end of the delay, a trial commenced with the extension of the setup lever and its cue light. A single press on the setup lever (fixed-ratio 1; FR1) activated the extension of the reward lever, which was armed on a VI10 schedule. After the rat completed the response requirement and received a single train of reward, a new ITI began. If the setup lever was not pressed, then the reward lever did not extend and a new ITI immediately commenced. Each test session had a total of 80 trials, which consisted of 40 primed and 40 non-primed trials that were presented randomly.

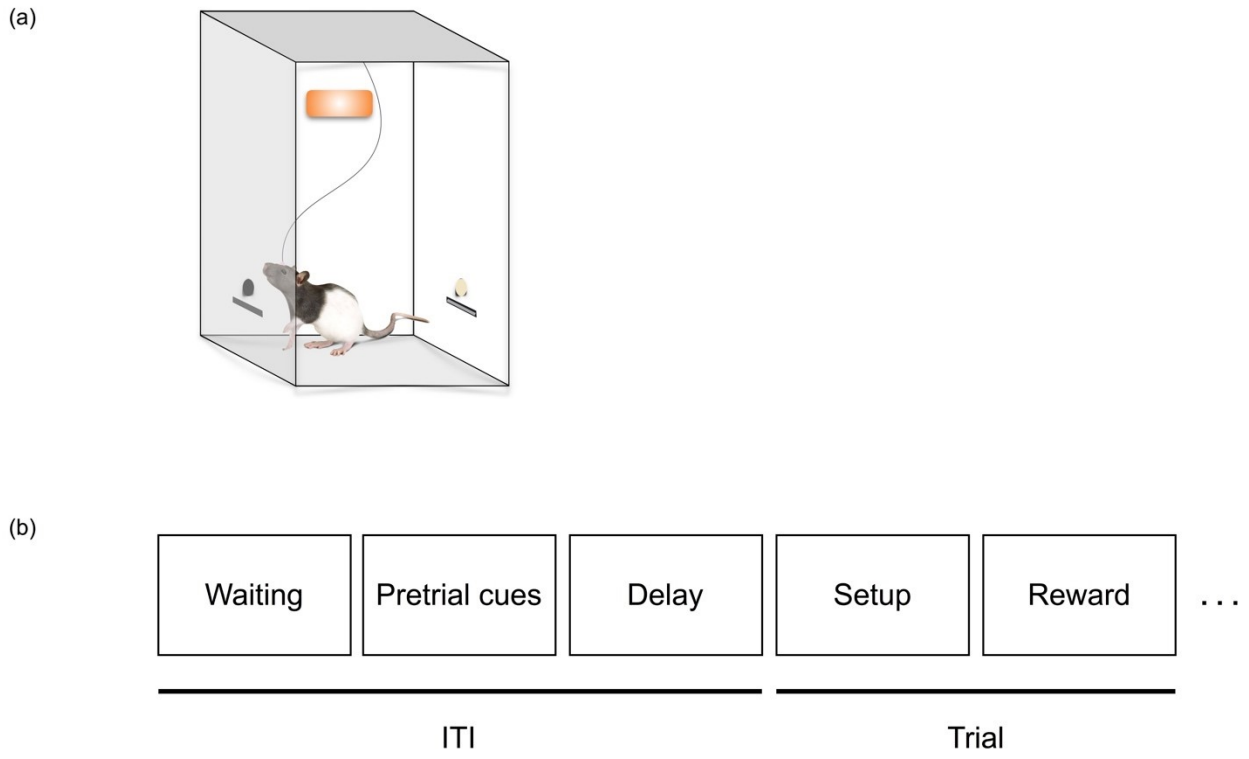

Figure 2. An image of the operant-conditioning chamber and a schematic of events during an ITI and a single trial. (a) The operant-conditioning chamber contains a setup lever (left) and a reward lever (right). (b) Preceding a trial was an ITI, which started with a waiting period. This was followed by a pretrial cues period, during which cues were delivered to signal that the start of a trial was approaching. On primed trials, priming stimulation was delivered during the pretrial cues period. This was followed by a delay and then the extension of the setup lever marked the start of a trial. A single press on the setup lever activated the extension of the reward lever located at the opposite side of the chamber. Completion of the response requirement on the reward lever delivered rewarding brain stimulation and initiated the start of a new ITI. The duration of each event within an ITI and trial is mentioned in the method section.

2.5 Statistical Analyses

For each rat, data were analyzed for each priming condition (primed, non-primed). Means were calculated for the cumulative response and response rate measures. Analyses and graphs were conducted using custom-written MatLab scripts (The Mathworks, Natick, MA).

Cumulative Response. The number of lever presses was calculated for each one-s time bin of each trial and cumulated. Cumulative responses between the primed and non-primed trials were compared at the 10-s time bin. This is because on a VI10 schedule, on average, a reward could be earned within 10 s of a trial.

Response Rate. The number of lever presses was calculated for each one-s time bin of each trial. These response frequencies were divided by the number of trials in which that time bin was sampled to yield the mean response per trial for each time bin. These means were multiplied by 60 to obtain the mean response rate per minute.

Confidence Intervals. Bootstrapping (Efron & Tibshirani, 1986) was used to determine means and their surrounding confidence intervals (CI). Data were randomly sampled with replacement to generate 500 re-sampled datasets. The upper and lower 2.5% of the distribution were defined as the bounds of a 95% CI. Non-overlap of the 95% CIs was used as the criterion for a statistically reliable effect between the primed and non-primed trials.

2.6 Procedures: Experiments 2 & 3

Training. Rats were each screened to determine which electrode, electrical current, and pulse frequency promoted vigorous lever pressing with minimal to no motor effects. The rats in these experiments responded for currents between 320 μA to 400 μA. The maximum pulse frequency of the reward stimulation ranged from 266 pps to 320 pps. The pulse frequency of the priming stimulation was lower, ranging from 198 pps to 222 pps. The consecutive trains of priming stimulation produced motor effects, so reducing the pulse frequency served to minimize those motor effects. The settings determined for each rat were used throughout the subsequent experiments.

Experiment 2 differed from experiment 1 in that a ratio schedule was used in lieu of a VI schedule. The rewarding stimulation consisted of a single 0.5-s train of 0.1-ms cathodal pulses. Once the operant behavior was stable, rats were trained in a testing procedure comparable to that in experiment 1. Rats learned to press a setup lever armed on a FR1 schedule that did not deliver

reward but instead activated the extension of a reward lever located at the opposite side of the operant-conditioning chamber. Here, the reward lever was armed on a ratio schedule.

To obtain curves expressing response rate as a function of reward strength, the response requirement was held constant at FR2 for all trials while the strength of the reward was systematically decreased. To obtain curves expressing response rate as a function of effort cost, the response requirement was progressively increased while the strength of the reward was held constant at a high value.

Responding on VI schedules tends to steady and slow; alterations in response rate have little or no effect on the rate at which rewards are delivered. In contrast, the rate of reward delivery is directly proportional to response rate on FR schedules. Thus, we expected that response rates would be more sensitive to the strength and cost of reward when a ratio schedule was in effect.

Testing. Rats initially underwent a warm-up session, during which they pressed a single lever for rewarding brain stimulation. A trial started with a 30-s ITI, during which the levers were retracted. The house light remained off during the first 15 s of the ITI. This waiting period was followed by pretrial cues signaling the start of a trial, which lasted for 10 s. Cues included flashing of the amber house light (10 cycles of 0.5s on, 0.5s off) and delivery of priming stimulation. Rats received 10 (high priming) or two (low priming) separate 0.5-s trains of 0.1 ms cathodal pulses, delivered at a rate of one train per second. On high-priming trials, primes were delivered at the start of the pretrial cue period, 15 s into the ITI. On low-priming trials, primes were delivered eight s into the pretrial cues period, or 23 s into the ITI. At the end of the pretrial cues period, the house light turned off and the five-s delay started. A trial began with the extension of the setup lever. A single response on the setup lever activated the extension of the reward lever, which was armed on a ratio schedule. Completion of the response requirement on the reward lever earned the rat a single train of reward, and a new ITI started. See Figure 1 for schematic of events in an ITI and a trial.

A set of 15 trials was called a block of trials (Figure 3a). The reward strength or cost remained constant within a block. Across blocks of trials, the strength or cost of the reward was varied systematically (Figure 3b & c). For example, to measure response curves as a function of reward strength, the pulse frequency was systematically decreased after every block of trials. Each test session consisted of 10 blocks of trials, totaling 150 trials. During a single test day, rats

underwent one session in the high-priming condition and a second session in the low-priming condition. The session order of the priming conditions was counterbalanced across days.

Previous studies that measured response curves as a function of reward strength or cost delivered priming stimulation prior to the start of each trial (Arvanitogiannis & Shizgal, 2008; Trujillo-Pisanty *et al*., 2012; Solomon *et al.*, 2015). In those studies, the strength or cost of reward changed following each trial. In this experiment, the strength or cost of the reward changed after a block of trials. Two stimulation trains served as primes in the low-priming condition rather than no stimulation at all. This was done in the hope that the low-priming stimulation would help motivate rats to sample the lever in the next trial so they could learn the new reward strength or cost.

Experiment 2a: Rate-Cost Curve. To measure curves expressing response rate as a function of reward cost, the ratio requirement was increased while the pulse frequency was held at a constant high value. The first block of trials started at the lowest cost (FR2) and increased across the blocks until the highest cost was reached (FR30). Data from the first block of trials and the first trial of each block served as a warmup block and a learning trial, respectively, and were excluded from analyses (Figure 3a). The lowest cost was in effect in both the first and second blocks; the cost was then increased in each subsequent block (Figure 3b). At least 10 test sessions were collected for each priming condition. Test sessions lasted approximately three hours.

Experiment 2b: Rate-Frequency Curve. To measure curves expressing response rate as a function of reward strength, the pulse frequency was systematically decreased and the response requirement was set to FR2. The first block of trials consisted of a highest pulse frequency and decreased across the blocks. Data from the first block of trials and the first trial of each block served as a warmup block and a learning trial, respectively, and were excluded from analyses (Figure 3a). The highest pulse frequency was in effect in both the first and second block; the pulse frequency was then reduced in equal proportional steps across the remaining blocks (Figure 3c). The step size between the pulse frequencies was adjusted for each rat to achieve a sigmoidal rate-frequency curve. The step sizes used in this experiment ranged from 0.08 to 0.18 log units. The curve was aimed to consist of nine different pulse frequencies that demonstrated maximal performance, decreasing performance, and no performance. At least 10 test sessions were collected for each priming condition. Test sessions lasted approximately two to three hours.

Experiment 3: Pimozide & Rate-Cost Curve. Pimozide, a D2R antagonist, was administered prior to obtaining curves that express response rate as a function of reward cost. Pimozide (0.1, 0.2, or 0.5 mg/kg; Sigma-Aldrich, St. Louis, MO) was dissolved in nanopure water. These doses were chosen based on previous experiments with electrical brain stimulation and pimozide (Atalay & Wise, 1983; Trujillo-Pisanty *et al.*, 2014). Test procedures were similar to those used in experiment 2a, with the exception that vehicle or one of the three doses of pimozide was administered i.p. three hours prior to the test session. Tests were conducted in three-day cycles consisting of a vehicle day, drug day, and washout day (Figure 3d). The session order of the priming conditions was counterbalanced across the three-day cycles. Tests were first conducted with the lowest dose of pimozide (0.1 mg/kg). Rats received each dose of pimozide at least 10 times in 10 separate tests. At least 10 test sessions were collected for each dose of pimozide before the next, higher dose was administered.

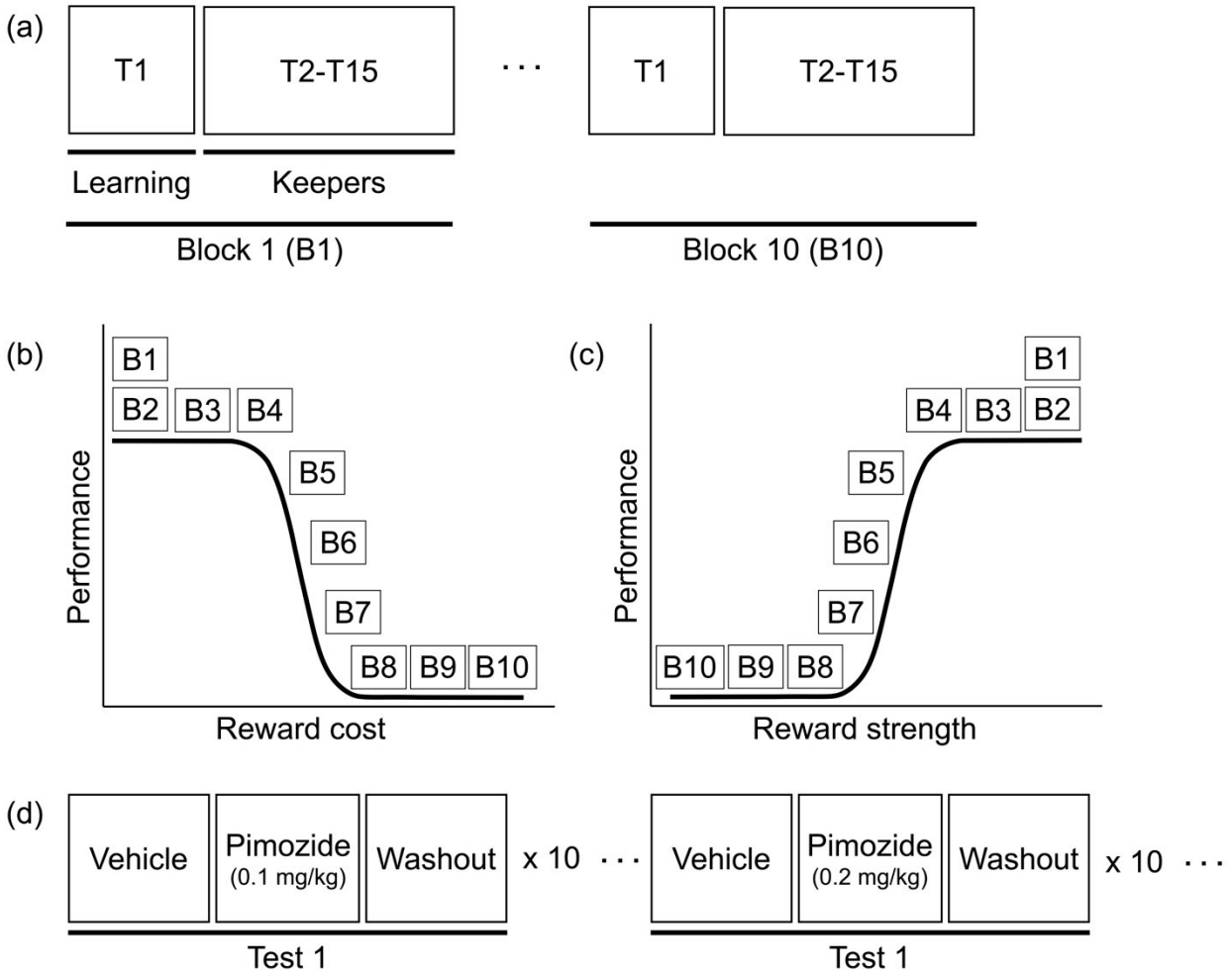

Figure 3. Schematic of procedures in experiments 2 and 3. (a) A block of trials consisted of 15 trials. The first trial (T1) was considered a "learning" trial and was not included in the analyses. The remaining 14 "keeper" trials (T2-T15) were analyzed. The reward strength or cost remained constant within a block. (b) In a rate-cost curve, the reward strength was set to a high value and the cost systematically increased across blocks. (c) In a rate-frequency curve, the cost was constantly low and the reward strength systematically declined across blocks. The first and second block of trials (B1 & B2) are in effect the same. However, B1 served as a warmup block and its data were not included in the analyses. (d) In experiment 3, tests were conducted in three-day cycles consisting of a vehicle day, drug day, and washout day. A single dose of pimozide was administered in at least 10 separate tests. After one dose was completed, new tests were conducted with a higher dose.

2.7 Statistical Analyses

For each rat, data were analyzed for each type of curve (rate-cost, rate-frequency), each priming condition (high or low), and drug dose (0, 0.1, 0.2, 0.5 mg/kg). The mean and median were calculated for the reward rate measure. Data were analyzed and graphs were plotted using custom-written MatLab scripts (The Mathworks, Natick, MA).

Reward Rate. Reward rate is the inverse of the total time (s) elapsed between the start of the trial and the delivery of the reward. Higher reward rates reflect more vigorous reward pursuit. This transformation is analogous to the running speed measure reported in Gallistel's investigations on the priming effect of electrical brain stimulation (Gallistel *et al.*, 1974; Stellar & Gallistel, 1975; Wasserman *et al.*, 1982).

Confidence Intervals. Bootstrapping (Efron & Tibshirani, 1986) was used to determine mean and median reward rates and their surrounding CIs. Trials in each block were randomly sampled with replacement to generate 1000 re-sampled datasets. Non-overlap of the 95% CIs was used as the criterion for a statistically reliable effect between the high- and low-priming conditions.

Distributions. The distribution of the data was visualized with violin plots based on kernel-density estimation (KDE). This is a non-parametric method for estimating the probability density function of a random variable, such as response speed. In contrast to traditional statistics, KDE addresses the data smoothing problem without prior parametric assumptions (e.g., normality). Instead, KDE creates smooth distributions based on a given sample of data.

Cliff's Delta. Visual inspection shows that the speed measure is both skewed and bimodal. Thus, a non-parametric analysis of effect size was employed. Cliff's delta is an effect size measure used for ordinal data that does not require assumptions about the distribution of the data. A Cliff's delta value near one indicates that high priming produced reliably faster response speeds compared to low priming. No difference between the high- and low-priming medians would yield a Cliff's delta value of zero. Bootstrapping (Efron & Tibshirani, 1986) with replacement was used to calculate both Cliff's delta and its surrounding 95% CI.

Difference ratio. A statistic was developed to assess the magnitude of the difference between speed measures obtained in the high- and low-priming conditions. The ratio of the difference between the two group medians was first calculated by means of bootstrapping with replacement (1000 resampled medians for both the high and no priming conditions). The

difference between the resampled medians was then normalized by the resampled grand median to yield the median difference ratio.

Criteria for a priming effect. A two-criterion approach was used to determine whether the results demonstrate a reliable and meaningful behavioral difference between the high- and low-priming conditions. The first criterion was a Cliff's delta greater than zero surrounded by a 95% CI excluding zero. The second criterion was a median difference ratio equal to or greater than .10 surrounded by a 95% CI excluding zero. A difference that met the criterion for Cliff's delta but not the median-difference-ratio criterion was considered statistically reliable but too small to be regarded as meaningful.

3. Results

3.1 Experiment 1: Measuring the Priming Effect of Electrical Brain Stimulation

Analyses of mean cumulative responding across the trial duration for each rat showed that the priming effect of electrical brain stimulation was observed in some, but not all, rats (Figure 4). Of the nine rats tested, three of them showed greater responding on the primed trials compared to the non-primed trials, which constitutes as a priming effect (Figure 4a). The remaining six rats showed no priming effect (Figure 4b) or a debatably trivial priming effect (Figure 4c).

The transient nature of the priming effect of electrical brain stimulation was observed when response rates were calculated across the trial duration (Figure 4d). The priming effect was observed during the first four s to 14 s of the trial duration. After these initial periods of higher responding on the primed trials, responding declined to levels similar to those in the non-primed trials. Thus, these data demonstrate that the priming effect decays with time.

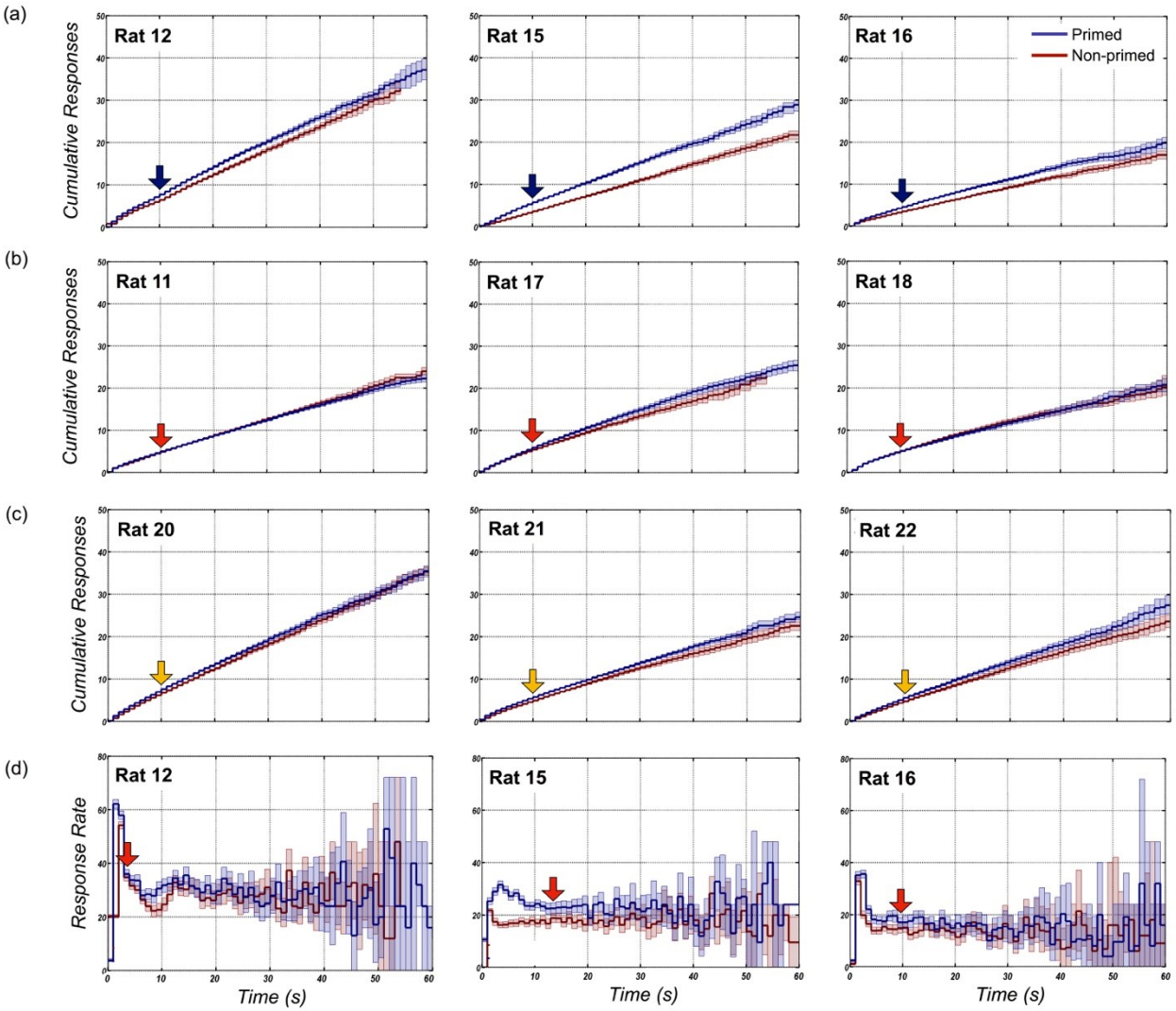

Figure 4. Priming elicits a transient increase in responding on a VI10 schedule in some, but not all, rats. In the cumulative response graphs, (a) blue arrows indicate a reliable priming effect, (b) red arrows indicate no priming effect, and (c) orange arrows indicate a debatably trivial priming effect. (d) Response rate graphs of rats that demonstrated a reliable priming effect. Red arrows indicate the time when the priming effect decays. Sold lines represent means and shaded regions represent 95% CIs. Non-overlap of the 95% CIs indicates a statistically reliable difference between the primed and non-primed trials.

3.2 Experiment 2a: Rate-Cost Curves

Analyses of the rate-cost curves showed that the mean reward rate was highest when the ratio requirement was low and the mean reward rate declined as the ratio requirement increased (Figure 5). High priming reliably increased the mean reward rate when the ratio requirement was low. As the ratio requirement increased, this difference shrunk and ultimately disappeared. Based on visual inspection, we observed that the slope of the rate-cost curve was steeper in response to high priming when the reward cost was inexpensive.

For the non-parametric analyses, the median reward rate at the lowest cost (e.g., FR2) was used to calculate effect sizes and difference ratios. The 95% CIs around Cliff's delta excluded zero for all eight rats, indicating a statistically reliable difference between the high- and low-priming conditions. Seven out of the eight rats met the .10 criterion for the median difference ratio. Thus, in one case, the difference between the high- and low-priming conditions was statistically reliable but was too small to be considered meaningful. The remaining rats showed a 12% to 24% difference between the high- and low-priming conditions relative to the grand median. Based on our two-criterion approach, seven out of eight rats showed reliable and meaningful increases in the median reward rate in response to high priming when the reward cost was inexpensive (Table 1).

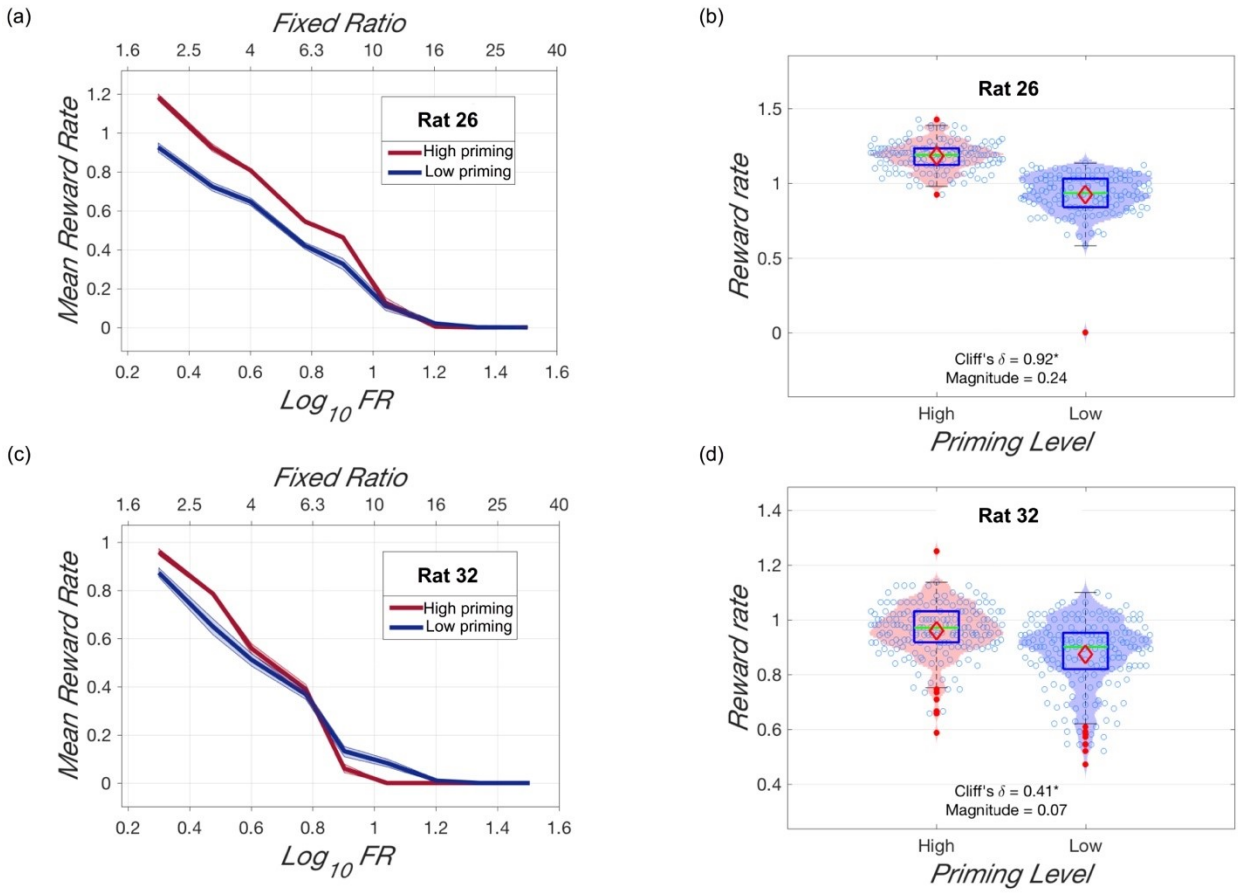

Figure 5. Rewards are obtained faster following high priming, and this depends on reward cost. (a) Effort-cost curve of rat 26 shows a (b) reliable and meaningful priming effect when the reward is inexpensive. (c) Effort-cost curve of rat 32 (d) does not show a meaningful priming effect when the reward is inexpensive. In the rate-cost curves (a & c), sold lines represent means and shaded regions represent 95% CIs. In the violin plots (b & d), blue open-dots represent individual data points, red-filled dots represent outliers, green lines represent the median, red diamonds represent the mean, and blue boxes represent the interquartile range.

Table 1

Reward Rates at the Lowest Reward Cost in the Rate-Cost Curve [a]

Rat No.	Median			Median Difference		Cliff's Delta	
	HP	LP	Grand	Ratio	95% CI	δ	95% CI
26	1.19	0.93	1.06	0.24	[0.20, 0.26]	0.92	[0.88, 0.95]
28	1.20	1.00	1.10	0.18	[0.15, 0.21]	0.70	[0.60, 0.78]
29	1.20	1.02	1.11	0.19	[0.15, 0.24]	0.57	[0.48, 0.66]
30	1.10	0.94	1.02	0.15	[0.11, 0.20]	0.47	[0.35, 0.58]
31	0.93	0.71	0.85	0.24	[0.19, 0.28]	0.73	[0.65, 0.81]
32	0.97	0.90	0.93	0.07*	[0.04, 0.09]	0.41	[0.31, 0.51]
33	0.76	0.63	0.70	0.19	[0.14, 0.23]	0.46	[0.35, 0.57]
34	0.94	0.83	0.89	0.12	[0.08, 0.17]	0.47	[0.36, 0.58]

[a] Curves were calculated from all trials in the counterbalanced high- and low-priming sessions.

* Did not meet the criteria for a reliable and/or meaningful priming effect.

Notes. HP = high priming. LP = low priming. Grey highlight emphasizes which rats did not show a reliable and/or meaningful priming effect.

3.3 Experiment 2b: Rate-Frequency Curves

Analyses of the rate-frequency curves showed that the mean reward rate was maximal when the reward was intense and then decreased as the reward weakened (Figure 6). High priming reliably increased the mean reward rate when the pulse frequency was high. That difference disappeared as the pulse frequency decreased. Based on visual inspection, the slope of the rate-frequency curve was steeper following high priming when the reward was intense.

Using non-parametric analyses, all eight rats showed that the median reward rate at the highest reward strength was greater following high priming compared to low priming. The 95% CIs of Cliff's delta excluded zero for all rats, indicating a statistically reliable difference between the high- and low-priming conditions. There was a 14% to 38% difference between the high- and low-priming conditions relative to the grand median, which indicates that all rats showed a meaningful priming effect (Table 2).

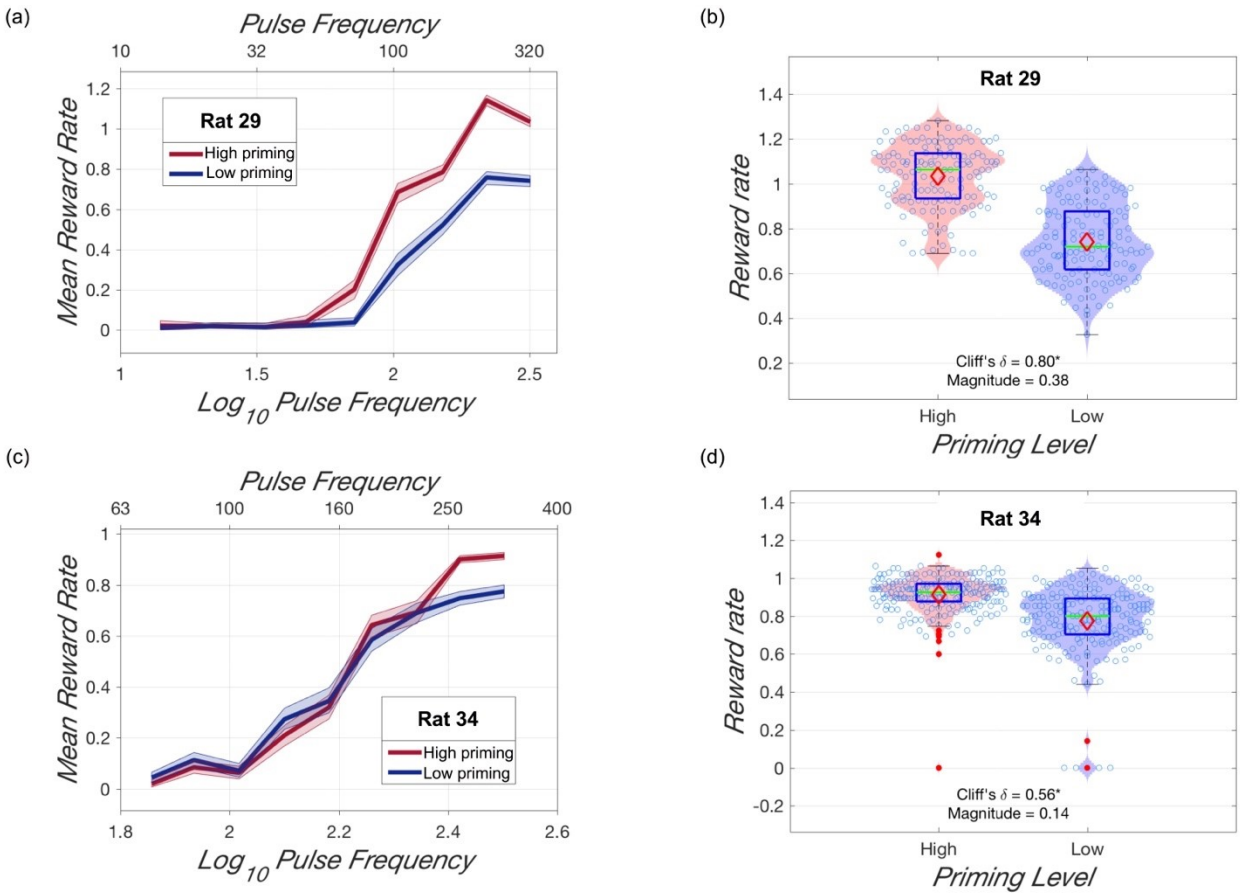

Figure 6. Rewards are obtained faster following high priming, and this depends on reward strength. (a) Rate-frequency curve of rat 29 show (b) a reliable and meaningful priming effect when the reward is intense. This rat showed the largest magnitude of difference between the high- and low-priming conditions at the strongest pulse frequency in the rate-frequency curve. (c) Rate-frequency curve of rat 34 shows (b) a reliable and meaningful priming effect. This rat showed the smallest magnitude of difference between the high- and low-priming conditions at the strongest pulse frequency in the rate-frequency curve. In the rate-frequency curves (a & c), sold lines represent means and shaded regions represent 95% CIs. In the violin plots (b & d), blue open-dots represent individual data points, red-filled dots represent outliers, green lines represent the median, red diamonds represent the mean, and blue boxes represent the interquartile range.

Table 2

Reward Rates at the Strongest Pulse Frequency in the Rate-Frequency Curves[a]

Rat No.	Median			Median Difference		Cliff's Delta	
	HP	LP	Grand	Ratio	95% CI	δ	95% CI
26	1.20	1.00	1.10	0.20	[0.18, 0.23]	0.96	[0.92, 0.98]
28	1.25	1.02	1.14	0.20	[0.18, 0.23]	0.80	[0.71, 0.87]
29	1.06	0.72	0.92	0.38	[0.31, 0.43]	0.80	[0.73, 0.86]
30	1.30	0.89	1.16	0.37	[0.32, 0.41]	0.87	[0.80, 0.92]
31	0.98	0.70	0.85	0.32	[0.29, 0.36]	0.90	[0.86, 0.94]
32	1.00	0.80	0.92	0.22	[0.18, 0.25]	0.63	[0.53, 0.71]
33	0.82	0.63	0.71	0.27	[0.24, 0.31]	0.65	[0.55, 0.74]
34	0.93	0.80	0.88	0.14	[0.11, 0.18]	0.56	[0.47, 0.64]

[a] Curves were calculated from all trials in the counterbalanced high- and low-priming sessions.

Notes. HP = high priming. LP = low priming.

3.4 Effect of Session Order

In experiments 2a and 2b, all trials were collectively analyzed, regardless of the session order of the priming conditions (Figures 5 & 6, Tables 1 & 2). Later, we assessed whether there was an effect of session order of the priming conditions (Figures 7 & 8). The rate-cost and rate-frequency curves were stable regardless of whether the high-priming condition was conducted as the first or second session (Figure 7b & 8b). This was not the case for the low-priming condition. The slopes of the rate-cost and rate-frequency curves were lower when the low-priming condition was conducted as the second session than when it was conducted first (Figure 7c & 8c). When the high-priming condition occurred first, subsequent maximal performance in the low-priming condition was impaired. This suppression in maximal performance on the following low-priming condition may have exaggerated or even mimicked a priming effect.

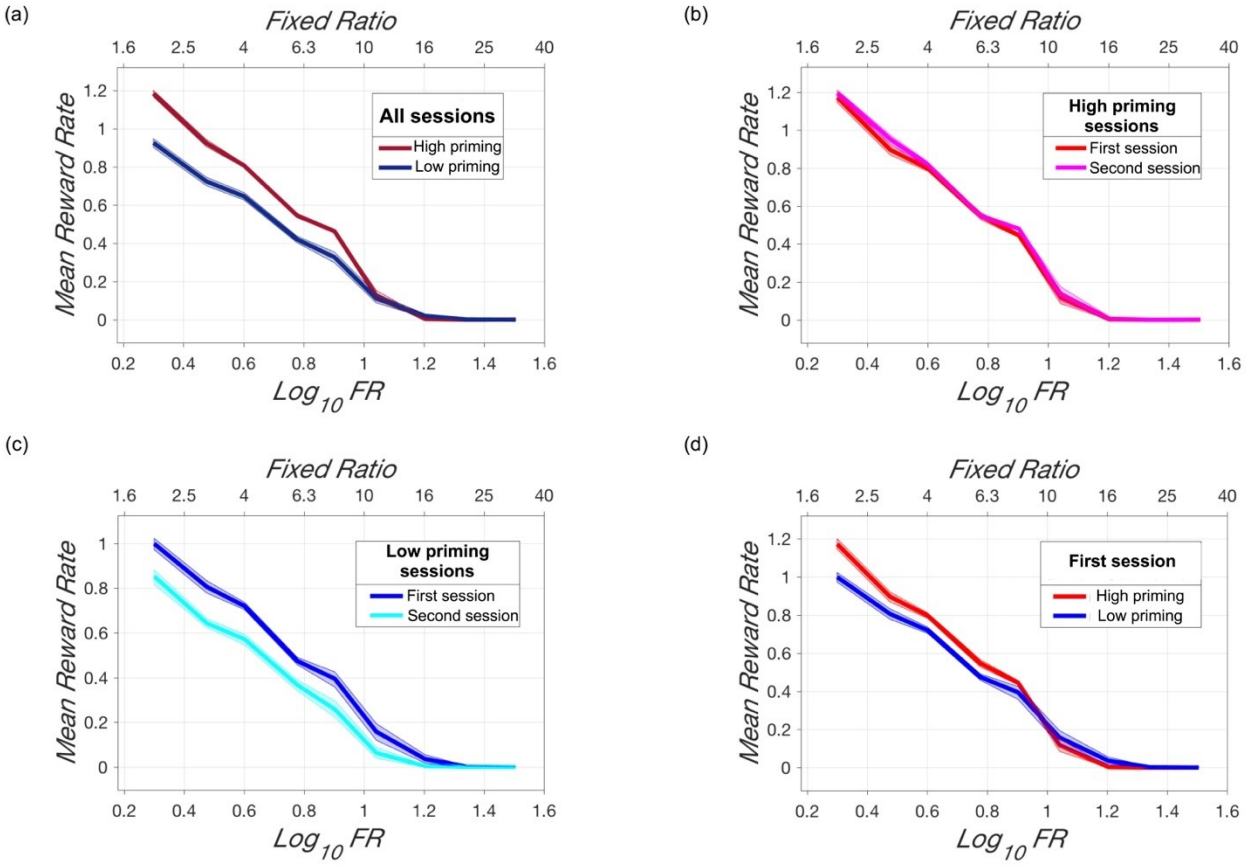

Figure 7. Effect of session order on rate-cost curves. (a) Initially, all trials from counterbalanced sessions were analyzed. (b) When only the high-priming sessions were analyzed, maximal performance is stable regardless of whether the high-priming session occurred before or after the low-priming session. (c) When the low-priming sessions were analyzed, maximal performance is blunted when the low-priming session was preceded by a high-priming session. (d) Due to this session order effect, the curves were re-analyzed to include data only from high- and low-priming sessions that were conducted as the first session.

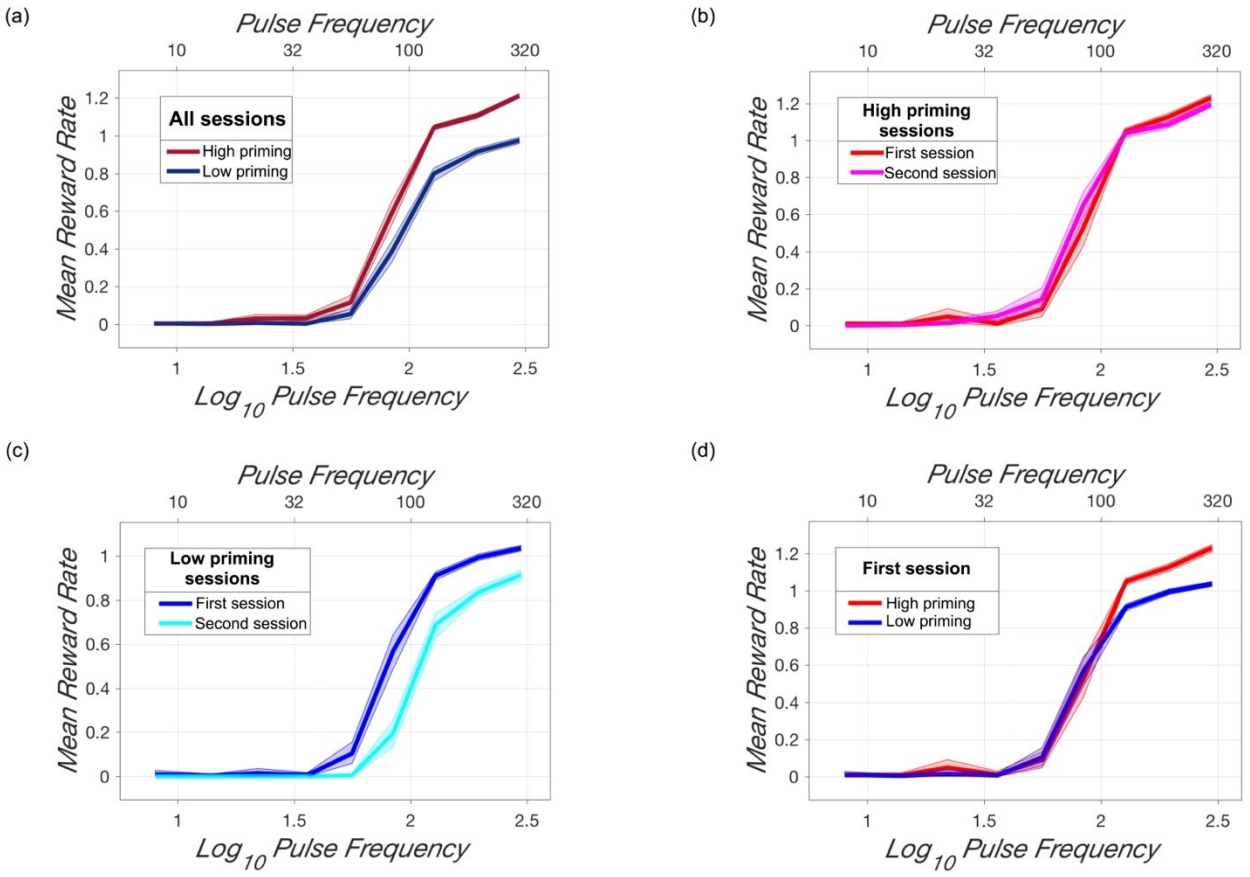

Figure 8. Effect of session order on rate-frequency curves. (a) Initially, all trials from counterbalanced sessions were analyzed. (b) When only the high-priming sessions were analyzed, maximal performance is stable regardless of whether the high-priming session occurred before or after the low-priming session. (c) When the low-priming sessions were analyzed, maximal performance is blunted when the low-priming session was preceded by a high-priming session. (d) Due to this session order effect, the curves were re-analyzed to include data only from high- and low-priming sessions that were conducted as the first session.

3.5 Re-Analyzed Rate-Cost Curves: First Session Only

To address the effect of session order, rate-cost curves were re-analyzed to include data only from the high- and low-priming conditions that were conducted as the first session. When this was conducted, analyses of the rate-cost curves showed that the mean reward rate was maximal when the reward was inexpensive and then decreased as the reward cost grew (Figure 9). There was a reliable difference in the mean reward rate between the high- and low-priming conditions when the reward cost was low. This difference dissipated as the reward cost increased. Based on visual inspection and non-overlap of the CIs, the slope of the rate-cost curve was steeper following high priming when the reward was inexpensive.

Using non-parametric analyses, two out of eight rats showed that the median reward rate at the cheapest reward cost was greater following high priming. The 95% CIs of Cliff's delta excluded zero for those rats, which indicates a statistically reliable difference between the high- and low-priming conditions. They also demonstrated a 13% to 16% increase in the median reward rate relative to the grand median following high priming, which indicates a meaningful priming effect (Figure 9, Table 3).

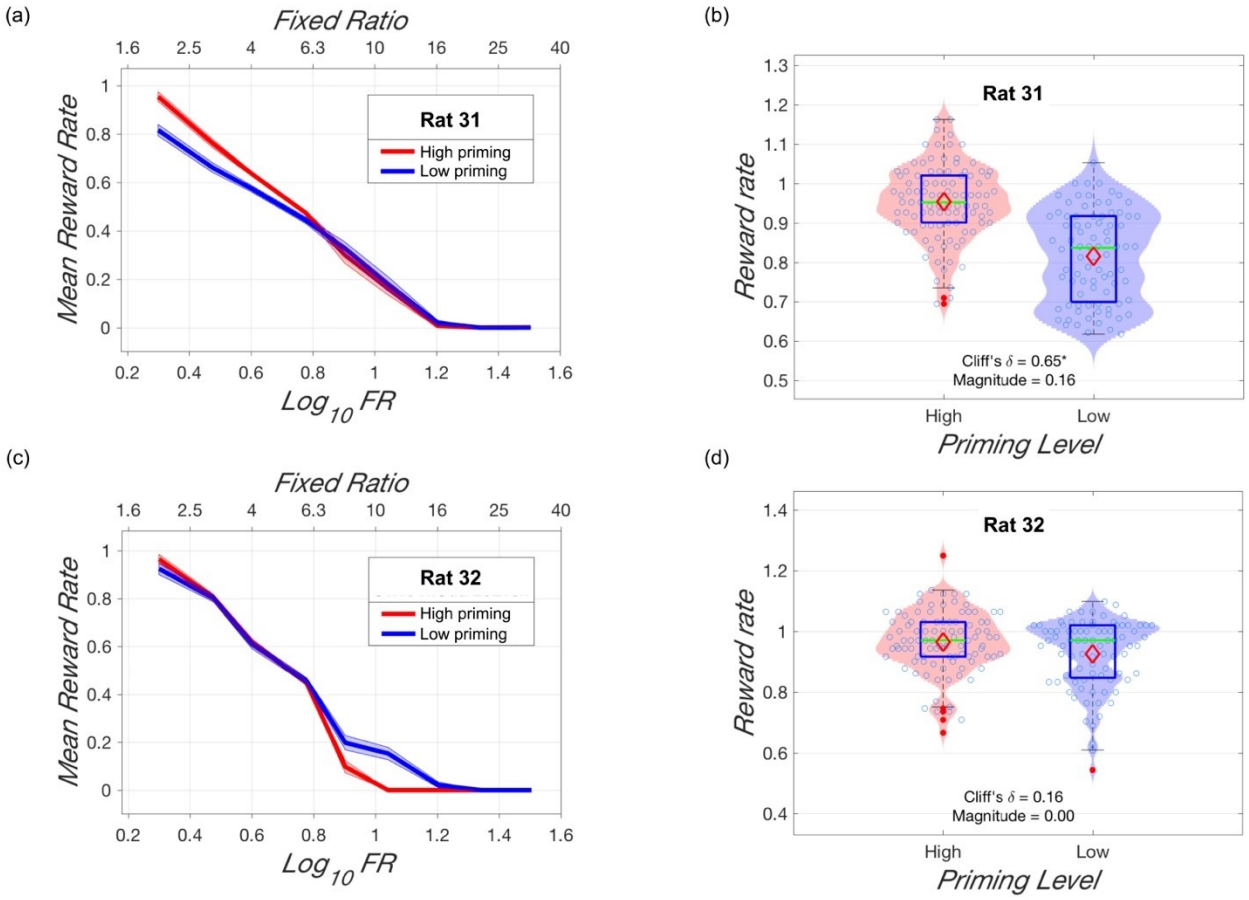

Figure 9. Re-analyzed reward rates show that the priming effect depends on reward cost, but there is greater variability in the incidence of a priming effect. (a) Rate-cost curve of rat 31 shows (b) a reliable and meaningful priming effect. (c) Rate-cost curve of rat 32 (b) does not show a reliable or meaningful priming effect. In the effort-cost curves (a & c), sold lines represent means and shaded regions represent 95% CIs. In the violin plots (b & d), blue open-dots represent individual data points, red-filled dots represent outliers, green lines represent the median, red diamonds represent the mean, and blue boxes represent the interquartile range.

Table 3

Re-analyzed Reward Rates at the Lowest Reward Cost in the Rate-Cost Curves[b]

Rat No.	Median			Median Difference		Cliff's Delta	
	HP	LP	Grand	Ratio	95% CI	δ	95% CI
26	1.16	1.03	1.06	0.13	[0.09, 0.17]	0.75	[0.62, 0.85]
28	1.18	1.09	1.10	0.09*	[0.03, 0.15]	0.45	[0.29, 0.60]
29	1.20	1.08	1.15	0.09*	[0.02, 0.14]	0.21	[0.09, 0.33]
30	1.11	1.05	1.09	0.05*	[0.02, 0.10]	0.30	[0.13, 0.46]
31	0.95	0.84	0.90	0.16	[0.11, 0.21]	0.65	[0.54, 0.75]
32	0.97	0.97	0.97	0.00*	[-0.03, 0.06]	0.16	[0.00, 0.31]*
33	0.77	0.75	0.75	0.04*	[0.01, 0.08]	0.17	[0.02, 0.33]
34	1.00	0.93	0.95	0.07*	[0.03, 0.11]	0.38	[0.24, 0.51]

[b] Curves were calculated from trials when the high- and low-priming sessions were conducted as the first session.

* Did not meet the criteria for a reliable and/or meaningful priming effect.

Notes. HP = high priming. LP = low priming. Grey highlight emphasizes which rats did not show a reliable and/or meaningful priming effect.

3.6 Re-Analyzed Rate-Frequency Curves: First Session Only

Due to the session order effect, rate-frequency curves were re-analyzed to include data only from the high- and low-priming conditions that were conducted as the first session. When this was conducted, analyses of the rate-frequency curves showed that the mean reward rate was maximal when the reward was intense and decreased as the reward weakened (Figure 10). There was a reliable difference in the mean reward rate between the high- and low-priming conditions when the pulse frequency was high. This difference reduced and eventually disappeared as the pulse frequency decreased. Based on visual inspection, the slope of the rate-frequency curve was steeper following high priming when the reward was intense.

Using non-parametric analyses, six out of eight rats showed that the median reward rate at the highest pulse frequency was greater following high priming. The 95% CIs of Cliff's delta excluded zero for those rats, which indicates a statistically reliable difference between the high- and low-priming conditions. They also demonstrated a 17% to 31% increase in the median reward rate relative to the grand median following high priming, which indicates a meaningful priming effect (Figure 10, Table 4).

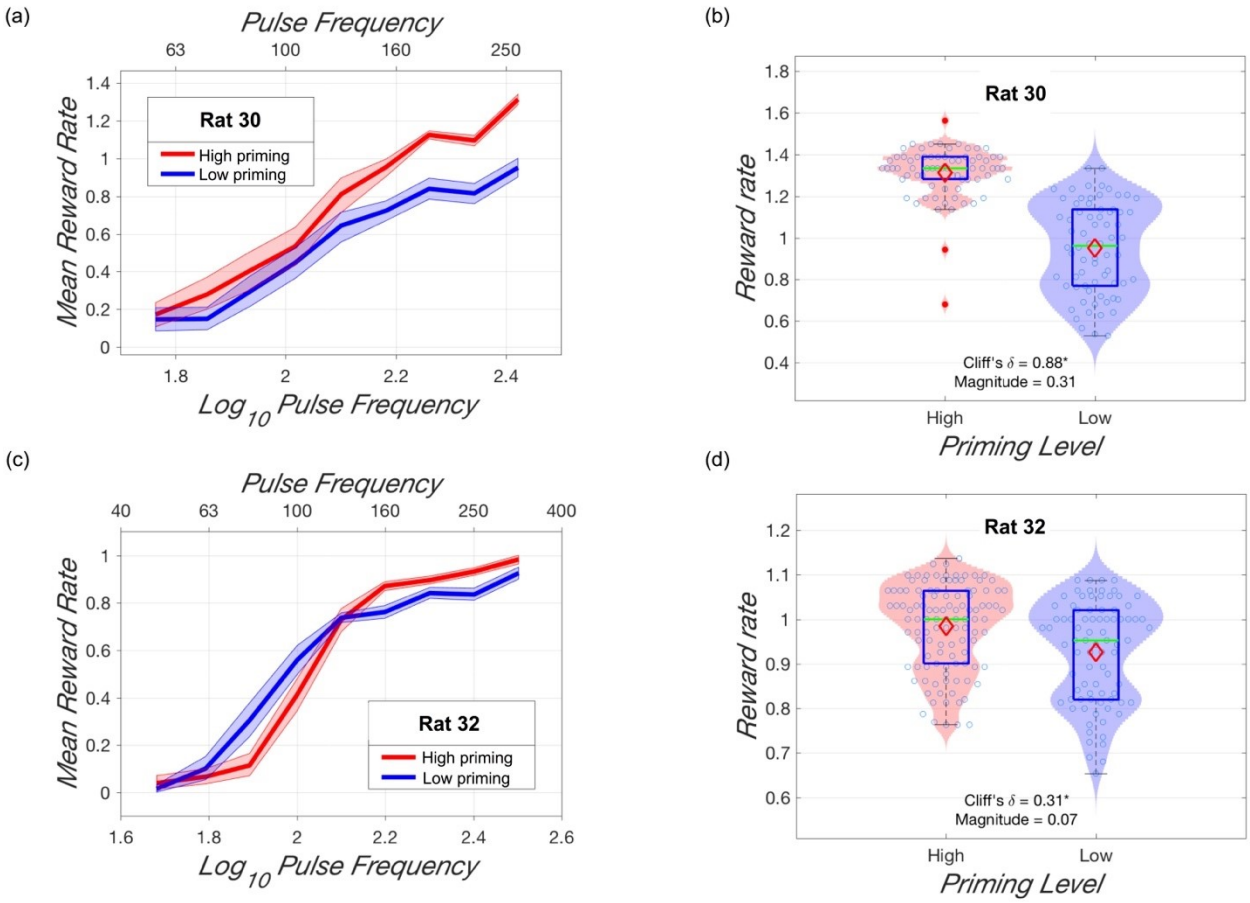

Figure 10. Re-analyzed reward rates show that the priming effect depends on reward strength, but there is greater variability in the incidence a priming effect. (a) Rate-frequency curve of rat 30 shows (b) a reliable and meaningful priming effect. (c) Rate-frequency curve of rat 32 (b) does not show a meaningful priming effect. In the rate-frequency curves (a & c), sold lines represent means and shaded regions represent 95% CIs. In the violin plots (b & d), blue open-dots represent individual data points, red-filled dots represent outliers, green lines represent the median, red diamonds represent the mean, and blue boxes represent the interquartile range.

Table 4

Re-analyzed Reward Rates at the Strongest Pulse Frequency in the Rate-Frequency Curves[b]

Rat No.	Median			Median Difference		Cliff's Delta	
	HP	LP	Grand	Ratio	95% CI	δ	95% CI
26	1.23	1.03	1.13	0.18	[0.16, 0.20]	0.93	[0.84, 0.99]
28	1.25	1.06	1.19	0.17	[0.14, 0.20]	0.75	[0.61, 0.87]
29	1.09	0.86	0.97	0.25	[0.19, 0.37]	0.80	[0.70, 0.88]
30	1.33	0.96	1.19	0.31	[0.22, 0.41]	0.88	[0.80, 0.94]
31	1.00	0.76	0.89	0.26	[0.22, 0.31]	0.84	[0.76, 0.90]
32	1.00	0.95	0.98	0.07*	[0.03, 0.10]	0.31	[0.17, 0.44]
33	0.83	0.66	0.78	0.21	[0.17, 0.27]	0.60	[0.48, 0.70]
34	0.94	0.87	0.90	0.09*	[0.05, 0.12]	0.48	[0.34, 0.61]

[b] Curves were calculated from trials when the high- and low-priming sessions were conducted as the first session.

* Did not meet the criteria for a reliable and/or meaningful priming effect.

Notes. HP = high priming. LP = low priming. Grey highlight emphasizes which rats did not show a reliable and/or meaningful priming effect.

3.7 Experiment 3: Pimozide & Rate-Cost Curve

To assess the role of dopamine transmission in the priming effect, rats were tested with three doses of pimozide (0.1 mg/kg, n = 4; 0.2 mg/kg, n = 8; 0.5 mg/kg, n = 5). All four rats tested with 0.1 mg/kg of pimozide showed a reliable and meaningful priming effect in response to either vehicle or pimozide. Their rate-cost curves showed that the mean reward rate was maximal when the reward was inexpensive and decreased as the reward cost grew (Figure 11). When the reward cost was low, the slope of the rate-cost curve was steeper following high priming. The difference between the high- and low-priming conditions relative to the grand median was 14% to 27% following vehicle and 13% to 32% following 0.1 mg/kg of pimozide (Table 5).

Eight rats were tested with vehicle before testing with 0.2 mg/kg of pimozide. Four showed a reliable and meaningful priming effect in response to vehicle. Their rate-cost curves showed that the mean reward rate was maximal when the reward cost was low and decreased as the reward cost grew (Figure 12). When the reward cost was low, the slope of the rate-cost curve was steeper following high priming. They showed a 12% to 13% difference between the high- and low-priming conditions relative to the grand median following vehicle (Table 6). Two out of four rats that showed a priming effect in response to vehicle also showed a priming effect in response to 0.2 mg/kg of pimozide. They showed a 15% difference between the high- and low-priming conditions relative to the grand median (Table 6).

Five rats were tested with vehicle before testing with 0.5 mg/kg of pimozide. Four rats showed a reliable and meaningful priming effect in response to vehicle. Their rate-cost curves showed that the mean reward rate was maximal when the reward cost was low and decreased as the reward cost became more expensive (Figure 12). When the reward cost was inexpensive, the slope of the rate-cost curve was steeper following high priming. They showed an 11% to 19% difference between the high- and low-priming conditions relative to the grand median following vehicle (Table 7). Of those four rats, three showed a reliable and meaningful priming effect following 0.5 mg/kg of pimozide. The difference between the high- and low-priming conditions relative to the grand median was 13% to 18% following 0.5 mg/kg of pimozide (Table 7).

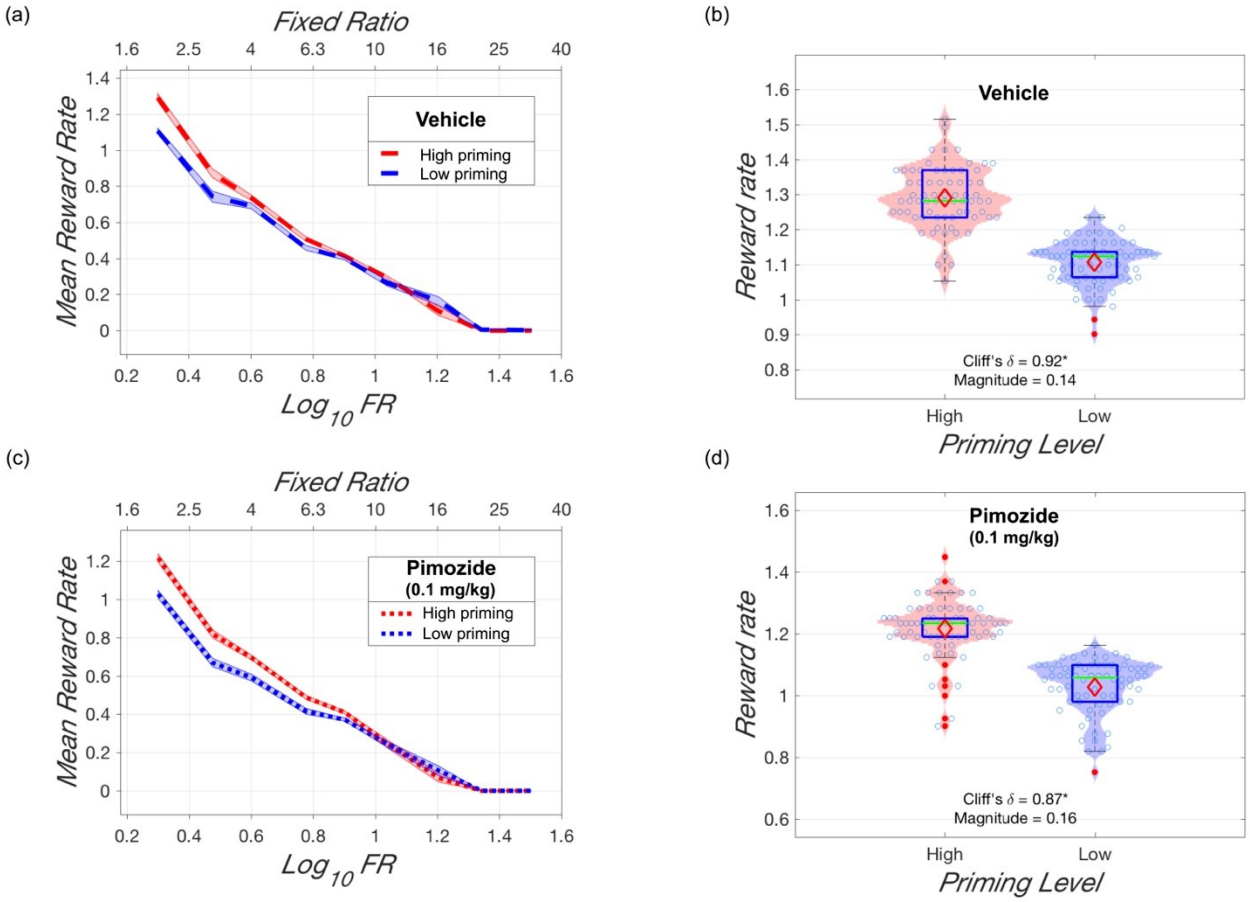

Figure 11. The priming effect of electrical brain stimulation persists following 0.1 mg/kg of pimozide. These are representative data from rat 26. (a) Rate-cost curve following (a) vehicle shows (b) a reliable and meaningful priming effect. (b) Rate-cost curve following 0.1 mg/kg of pimozide shows (c) a reliable and meaningful priming effect. In the effort-cost curves (a & c), broken lines represent means and shaded regions represent 95% CIs. In the violin plots (b & d), blue open-dots represent individual data points, red-filled dots represent outliers, green lines represent the median, red diamonds represent the mean, and blue boxes represent the interquartile range.

Table 5

Reward Rates at the Lowest Reward Cost in the Rate-Cost Curves[b] following Vehicle and 0.1 mg/kg of Pimozide

	Vehicle							Pimozide (0.1 mg/kg)						
Rat No.	Median			Median Difference		Cliff's Delta		Median			Median Difference		Cliff's Delta	
	HP	LP	Grand	Ratio	95% CI	δ	95% CI	HP	LP	Grand	Ratio	95% CI	δ	95% CI
26	1.28	1.12	1.19	0.14	[0.12, 0.17]	0.92	[0.84, 0.97]	1.23	1.06	1.12	0.16	[0.13, 0.19]	0.87	[0.76, 0.96]
28	1.28	1.10	1.19	0.15	[0.10, 0.19]	0.76	[0.63, 0.86]	1.20	1.06	1.13	0.13	[0.09, 0.18]	0.71	[0.57, 0.86]
29	1.10	0.85	0.95	0.27	[0.19, 0.35]	0.62	[0.47, 0.75]	1.01	0.84	0.89	0.19	[0.07, 0.28]	0.42	[0.24, 0.57]
30	1.37	1.05	1.16	0.27	[0.20, 0.30]	0.68	[0.54, 0.80]	1.35	1.02	1.06	0.32	[0.25, 0.36]	0.81	[0.64, 0.91]

[b] Curves were calculated from trials when the high- and low-priming sessions were conducted as the first session.

Notes. HP = high priming. LP = low priming. Grey highlight emphasizes which rats did not show a meaningful priming effect.

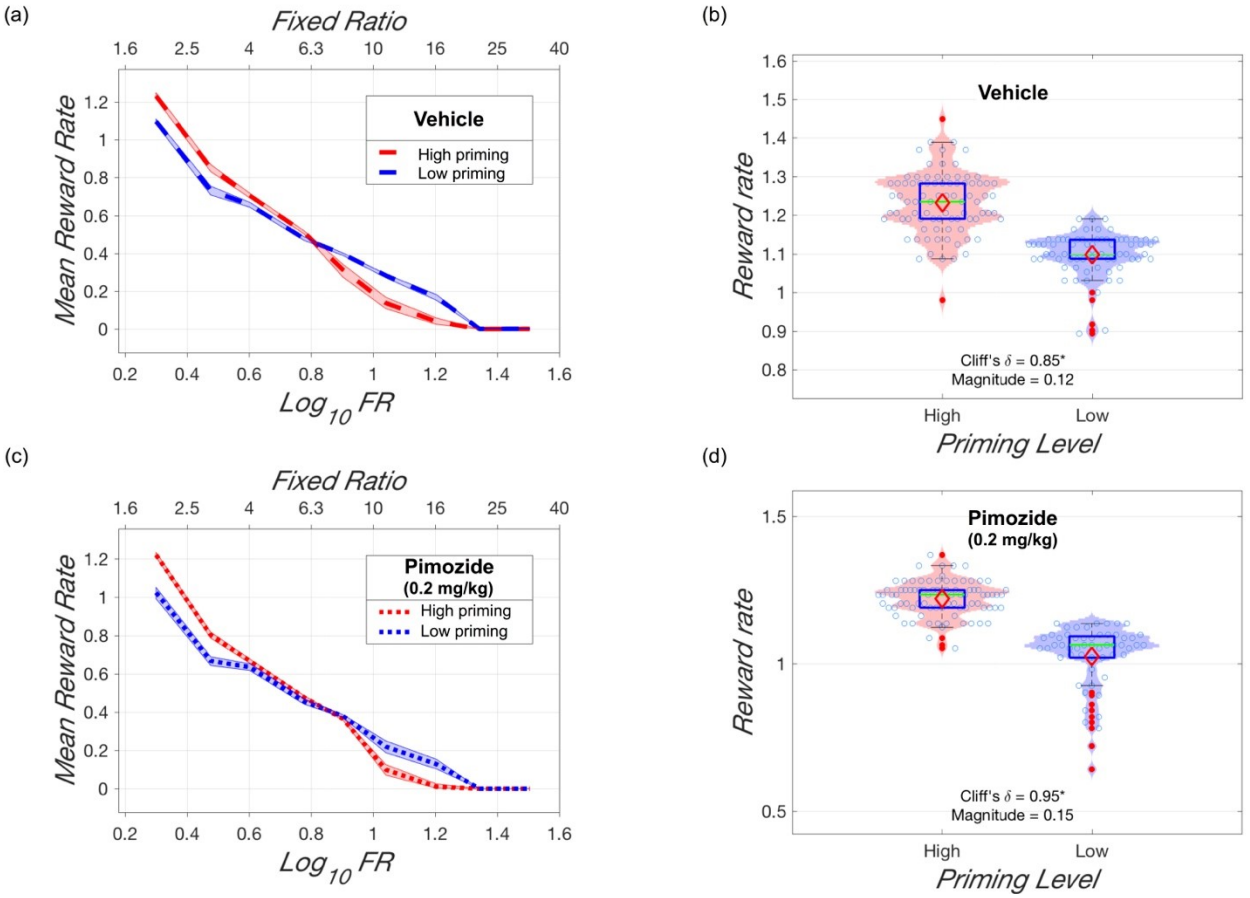

Figure 12. The priming effect of electrical brain stimulation persists following 0.2 mg/kg of pimozide. These are representative data from rat 26. (a) Rate-cost curve following vehicle shows (b) a reliable meaningful priming effect. (B) Rate-cost curve following 0.2 mg/kg of pimozide shows (c) a reliable meaningful priming effect. In the effort-cost curves (a & c), broken lines represent means and shaded regions represent 95% CIs. In the violin plots (b & d), blue open-dots represent individual data points, red-filled dots represent outliers, green lines represent the median, red diamonds represent the mean, and blue boxes represent the interquartile range.

Table 6

Reward Rates at the Lowest Reward Cost in the Rate-Cost Curves[b] following Vehicle and 0.2 mg/kg of Pimozide

	Vehicle							Pimozide (0.2 mg/kg)						
Rat No.	Median			Median Difference		Cliff's Delta		Median			Median Difference		Cliff's Delta	
	HP	LP	Grand	Ratio	95% CI	δ	95% CI	HP	LP	Grand	Ratio	95% CI	δ	95% CI
26	1.23	1.10	1.14	0.12	[0.07, 0.14]	0.85	[0.77, 0.92]	1.23	1.06	1.14	0.15	[0.11, 0.17]	0.95	[0.90, 0.98]
28	1.20	1.06	1.14	0.12	[0.09, 0.15]	0.67	[0.53, 0.79]	1.16	1.00	1.09	0.15	[0.10, 0.23]	0.73	[0.60, 0.85]
29	1.10	1.01	1.06	0.09*	[-0.03, 0.16]*	0.24	[0.05, 0.42]							
30	1.06	1.02	1.05	0.05*	[0.02, 0.09]*	0.38	[0.24, 0.52]							
31	1.00	0.88	0.95	0.13	[0.07, 0.18]	0.62	[0.48, 0.75]	0.95	0.90	0.93	.06*	[0.03, 0.12]	0.40	[0.23, 0.56]
32	0.94	0.89	0.92	0.05*	[0.02, 0.07]	0.23	[0.11, 0.35]							
33	0.85	0.75	0.82	0.13	[0.09, 0.19]	0.41	[0.29, 0.52]	0.84	0.76	0.82	.09*	[0.04, 0.13]	0.28	[0.16, 0.40]
34	0.98	1.00	0.98	-0.02*	[-0.06, 0.02]*	-0.15*	[-0.32, 0.03]							

[b] Curves were calculated from trials when the high- and low-priming sessions were conducted as the first session.

* Did not meet the criteria for a reliable and/or meaningful priming effect.

Notes. HP = high priming. LP = low priming. Grey highlight emphasizes which rats did not show a reliable and/or meaningful priming effect.

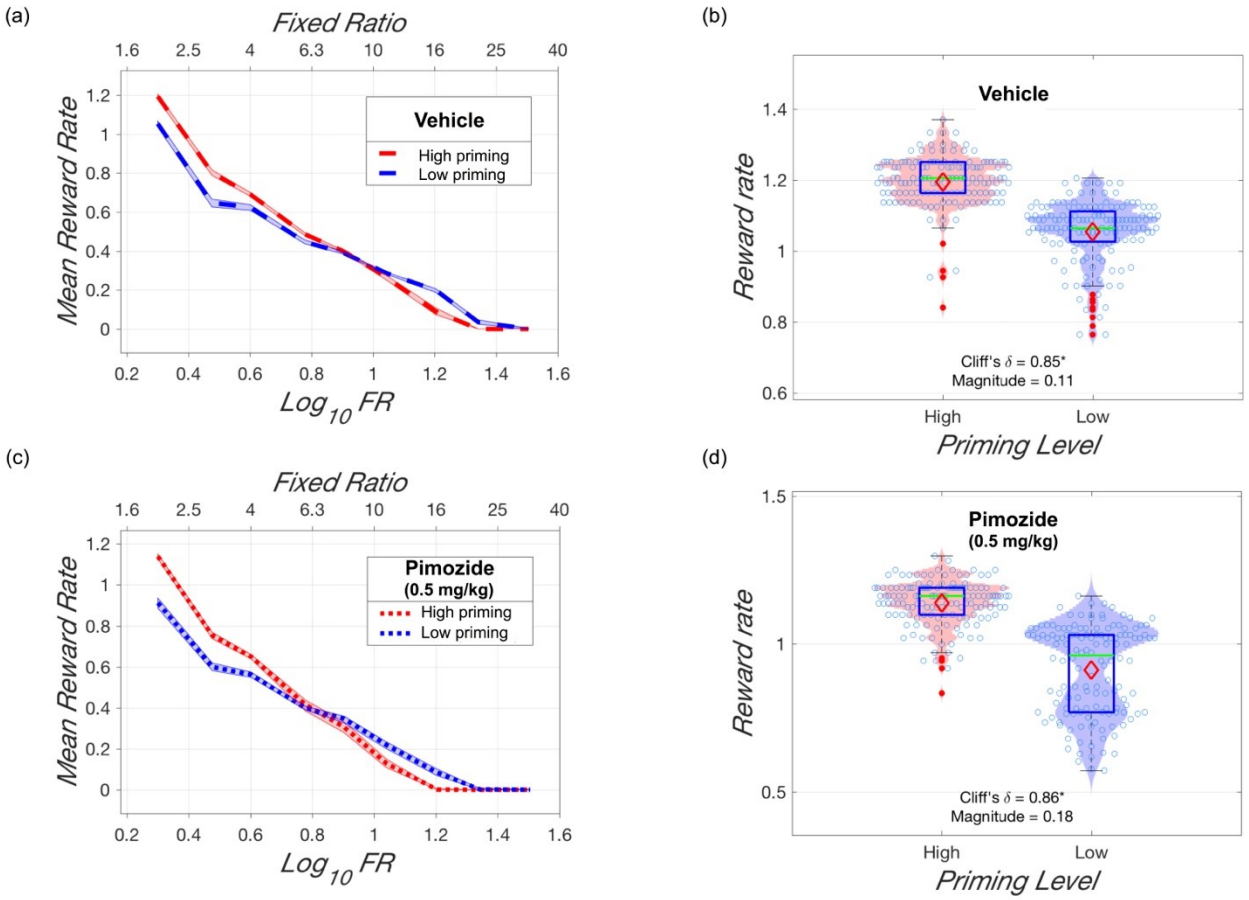

Figure 13. The priming effect of electrical brain stimulation persists following 0.5 mg/kg of pimozide. These are representative data from rat 26. (a) Rate-cost curve following vehicle shows (b) a reliable and meaningful priming effect. (B) Rate-cost curve following 0.5 mg/kg of pimozide shows (c) a reliable and meaningful priming effect. In the effort-cost curves (a & c), broken lines represent means and shaded regions represent 95% CIs. In the violin plots (b & d), blue open-dots represent individual data points, red-filled dots represent outliers, green lines represent the median, red diamonds represent the mean, and blue boxes represent the interquartile range.

Table 7

Reward Rates at the Lowest Reward Cost in the Rate-Cost Curves[b] following Vehicle and 0.5 mg/kg of Pimozide

	Vehicle							Pimozide (0.5 mg/kg)						
Rat No.	Median			Median Difference		Cliff's Delta		Median			Median Difference		Cliff's Delta	
	HP	LP	Grand	Ratio	95% CI	δ	95% CI	HP	LP	Grand	Ratio	95% CI	δ	95% CI
26	1.20	1.06	1.14	0.11	[0.09, 0.13]	0.85	[0.78, 0.91]	1.16	0.96	1.06	0.18	[0.13, 0.27]	0.86	[0.80, 0.91]
28	1.12	0.93	1.02	0.19	[0.14, 0.22]	0.70	[0.61, 0.79]	1.03	0.90	0.95	0.14	[0.11, 0.17]	0.51	[0.38, 0.63]
29	1.10	0.95	1.03	0.14	[0.08, 0.17]	0.48	[0.36, 0.59]	1.04	0.92	0.97	0.13	[0.07, 0.18]	0.44	[0.33, 0.55]
30	0.97	1.02	1.00	-0.05*	[-0.07, -0.02]*	-0.15*	[-0.25, -0.04]*							
32	0.90	0.86	0.88	0.04*	[0.02, 0.07]	0.37	[0.19, 0.54]							
33	0.83	0.72	0.78	0.13	[0.08, 0.19]	0.42	[0.30, 0.53]	0.78	0.76	0.78	0.03*	[-0.02, 0.09]*	0.10	[-0.04, 0.24]*

[b] Curves were calculated from trials when the high- and low-priming sessions were conducted as the first session.

* Did not meet the criteria for a reliable and/or meaningful priming effect.

Notes. HP = high priming. LP = low priming. Grey highlight emphasizes which rats did not show a reliable and/or meaningful priming effect.

4. Discussion

Receipt of a reward enhances motivation to work for more, and this phenomenon is called the priming effect of rewards. Motivation to pursue a reward is affected by variables such as the strength and cost of reward. When electrical brain stimulation is used as a reward, we showed that its priming effect depends on these two variables. Specifically, the priming effect of electrical brain stimulation is more likely to be observed when the reward is intense and inexpensive.

In experiment 1, a new method was developed for measuring the priming effect of electrical brain stimulation. After it was observed that only a third of the rats showed a priming effect, experiment 2 aimed to reduce the variability in the incidence of a priming effect by examining how priming is affected by reward strength and cost. A priming effect was consistently observed in the rate-frequency and rate-cost curves when all trials from the counterbalanced sessions were analyzed. However, an effect of session order of the priming conditions may have exaggerated or mimicked a priming effect. To address this issue, only the first sessions were analyzed. Although the number of rats that showed a priming effect decreased, we are more confident that the resulting priming effect is *bona fide*. Lastly, experiment 3 examined if the priming effect depends on D2R signaling. The results showed that the priming effect of electrical brain stimulation persists following dopamine receptor antagonism with pimozide.

4.1 Reward Strength & Effort Cost

Reward seeking is highest when the reward is intense and declines as the reward weakens (Edmonds & Gallistel, 1974; Miliaressis *et al*., 1986). Similarly, reward seeking is greatest when the reward cost is low and dissipates as the cost grows (Arvanitogiannis & Shizgal, 2008; Trujillo-Pisanty *et al.*, 2014). Since the priming effect of rewards is a boost in motivation to seek rewards, it was thought that priming would be affected by reward strength and cost.

We showed that the priming effect of electrical brain stimulation occurs when a reward is intense and the cost is low. No other studies have examined the effect of reward cost on priming. Edmonds and Gallistel (1974) conducted the only other study on whether the priming effect is affected by reward strength. They measured eICSS at varying reward strengths to produce a rate-frequency curve. Priming was found to enhance maximal performance for intense rewards, which is in accordance with the present study's results.

The findings from experiment 2 can explain the results from experiment 1. Only a third of the rats tested in experiment 1 showed a priming effect. There, only a single value of reward strength (178 pps) was used, which may have been perceived as too weak for some rats to elicit a priming effect. Although experiment 2 shows that a priming effect is more likely to be observed when the reward is intense, there was still a large amount of variability in the incidence of a priming effect. This is discussed in the following sections.

4.2 A Novel Method for Measuring the Priming Effect of Electrical Brain Stimulation

We developed a new method to measure the priming effect of electrical brain stimulation based on rates of lever pressing. To earn a reward, rats were required to press a setup lever to activate the extension of a second, separate reward lever that was armed on a pre-determined reinforcement schedule. During preliminary tests, only a single reward lever was used. It was observed that rats did not consistently start a trial in the same location and they performed superstitious behaviors, which added variability to the response latency and reward rate measures. Introduction of a setup lever required each rat to start a trial in the same location and it helped eliminate superstitious behaviors during operant conditioning.

The work performed on the setup and reward levers may be considered comparable to the work performed on the start box, alley, and goal box of the runway paradigm. In the runway paradigm, rats receive priming stimulation in a start box. They then travel to the end of an alley to reach a goal box that contains a lever that delivers rewarding brain stimulation when pressed (Gallistel *et al.*, 1974). Pressing the setup lever in our paradigm is similar to a rat exiting the start box in the runway paradigm. Afterwards, in our paradigm, the rat presses the reward lever to earn a reward. This is comparable to the rat traveling the distance of the alley to reach the goal box to lever press for rewarding brain stimulation. Thus, the paradigm used here was developed to be similar to the runway paradigm.

In experiment 1, the reward lever was armed on VI10 schedule. Only a third the rats tested showed a priming effect. A potential reason for this result is that a single value of reward strength (178 pps) was used for all rats. Due to variability in electrode placement, 178 pps of stimulation could have been intense for one rat but weak for another rat. Another potential reason is that the rate of reward delivery on VI schedules is little affected by changes in the rate of responding. In that sense, increases in response rate in the high-priming condition were largely futile.

To address these issues and in an effort to optimize our paradigm, operant responding was measured at varying reward strengths and costs in experiment 2. To vary reward strength, we determined the highest pulse frequency that promotes eICSS for each rat and produces minimal to no motor effects. Varying reward cost required using a different reinforcement schedule. In a preliminary test, a cumulative hold-down schedule was implemented because it has previously been used to measure performance as a function of reward strength and cost in the reward-mountain model (Arvanitogiannis & Shizgal, 2008; Hernandez *et al.*, 2010; Breton *et al.*, 2013; Trujillo-Pisanty *et al.*, 2014; Solomon *et al.*, 2015). Rats were required to depress a lever for four s to earn a reward. Results from our preliminary experiment showed an inconsistent priming effect (Ewusi-Boisvert, 2016). The cumulative hold-down schedule presents a similar problem to the VI schedule used in experiment 1: the rat has little control over how soon it can earn a reward. In contrast, in the runway paradigm, if the rat runs faster down the alley then it can obtain the reward sooner. Thus, in experiment 2, a ratio schedule was used because the rate of reward delivery in that schedule is related to the response rate.

After applying these changes in experiment 2, rate-cost curves showed that 25% of the rats tested demonstrated invigorated maximal responding following high priming (Table 3). On the other hand, rate-frequency curves showed that 75% of the rats tested demonstrated a boost in maximal responding following high priming (Table 4). These results indicate that our new method is far from optimal. Nevertheless, it was observed in some rats that priming invigorates responding when the reward is intense and inexpensive.

4.3 Variability in the Observation of the Priming Effect of Electrical Brain Stimulation

After refining the method for measuring the priming effect of electrical brain stimulation in experiment 2, the number of rats that showed a priming effect was still variable. In contrast, Reid *et al.* (1973) consistently showed a priming effect of electrical brain stimulation when using a runway paradigm. The magnitude of the priming effect varied among rats; nevertheless, they reported a 100% incidence of a priming effect.

We observed a priming effect of electrical brain stimulation in 88% to 100% of the rats when all trials from the counterbalanced priming conditions were analyzed (Tables 1 & 2). However, an effect of session order of the priming conditions biased those results. Maximal performance was stable regardless of whether the high-priming session occurred before or after the low-priming session (Figure 7b & 8b). However, maximal performance was blunted when

the high-priming session occurred prior to the low-priming session (Figure 7c & 8c). This indicates that the session order effect inflated or falsely produced a priming effect when all sessions were analyzed. When data were analyzed from the high- and low-priming conditions that were conducted as the first session of the day, incidence of a priming effect varied from 25% to 75% of the rats (Tables 3 & 4).

A plausible explanation for this result is that stimulation during the high-priming condition may have depleted dopamine levels. Hernandez *et al.* (2006) investigated the effect of prolonged MFB stimulation on tonic dopamine levels in the nucleus accumbens using *in vivo* microdialysis. Rats stimulated at a rate of five trains per minute showed elevated dopamine levels that plateaued during a two-hour stimulation period. When 40 trains were delivered per minute, dopamine levels increased but then sharply decreased after approximately 30 minutes of stimulation. Dopamine depletion has been related to reduced motivation to seek rewards (Cousins *et al.*, 1996; Salamone *et al.*, 2001, 2009). Therefore, it is possible that high priming stimulation depleted dopamine levels and consequently suppressed maximal performance in the following low-priming condition.

4.4 The Priming Effect of Electrical Brain Stimulation & Pimozide

It is well-established that dopamine transmission is important for reward and motivation. Pimozide has been shown to attenuate eICSS but does not abolish the capacity to perform (Franklin & McCoy, 1979). Thus, the rewarding effect of brain stimulation depends on D2R signaling. Dopamine depletion and dopamine receptor antagonism attenuate willingness to work for reward (Aberman & Salamone, 1999; Salamone *et al.*, 2001). Based on these studies evidence, it would be expected that the priming effect of rewards is mediated by dopamine transmission.

On the contrary, Wasserman *et al.* (1982) showed that primed rats continue to run faster to the end of the alley to lever press for rewarding brain stimulation following administration of high doses of pimozide. Similarly, when rate-cost curves were measured in experiment 3, a majority of the rats that showed a priming effect in response to vehicle also showed a priming effect following pimozide. However, pimozide binds to dopamine D2Rs, D3Rs, and serotonin $5HT_7$ receptors. Thus, the involvement of dopamine transmission in the priming effect cannot be ruled out based on studies with pimozide.

4.5 Conclusion

In conclusion, the priming effect of electrical brain stimulation is sensitive to reward strength and cost. With our new method that measures the priming effect based on rates of lever pressing, we showed that the priming effect is more likely to be observed when the reward is intense and inexpensive. In accordance with previous research, the priming effect of electrical brain stimulation is not blocked by pimozide. Nevertheless, additional studies are needed to provide more compelling evidence for whether the priming effect of electrical brain stimulation depends on dopamine transmission.

Chapter 3: Dopamine D2-like Receptor Antagonism Does Not Block the Priming Effect of Electrical Brain Stimulation

Abstract

Electrical brain stimulation elicits a rewarding effect and a priming effect. The rewarding effect is expressed as the proclivity to seek electrical brain stimulation. The priming effect is expressed as invigoration of that behavior following receipt of an intense reward. Reward and motivation have both been linked with dopamine transmission. However, there is evidence that indicates that the priming effect of electrical brain stimulation may not rely on dopamine transmission. Here, we used a novel method to investigate whether dopamine transmission is necessary for the priming effect of electrical brain stimulation. In experiment 1, a behavioral design was modified from a previous study (Chapter 2) in the hope of obtaining a more consistent priming effect. To assess if the priming effect of electrical brain stimulation depends on dopamine transmission, a dopamine D2 receptor family (D2R) antagonist, eticlopride, was administered at two doses (0.1 mg/kg, 0.05 mg/kg) in experiment 2. The method used in the present study yielded a more consistent priming effect than in the previous study (Chapter 2). Furthermore, we found that the priming effect of electrical brain stimulation persists following dopamine receptor antagonism. These results indicate that although dopamine transmission is important role for reward and motivation, we provide evidence that the priming effect does not depend on D2R signaling.

1. Introduction

Electrical brain stimulation produces a rewarding effect and a priming effect (Wasserman *et al.*, 1982). These effects have been studied using a runway paradigm that consists of a start box, an alley, and a goal box. Rats are primed with non-contingent brain stimulation in the start box. Following a delay, a start door opens to allow rats to travel to the goal box located at the end of the alley, which contains a lever that delivers brain stimulation when pressed. The rewarding effect of the response-contingent brain stimulation received in the goal box is expressed as the proclivity of the rat to run down the alley and the value it assigns to the stimulation available there. The priming effect of the non-contingent start-box stimulation is expressed as a transient increase in running speed to lever press for rewarding brain stimulation in the goal box.

The priming effect discussed here is different from the identically named priming effect observed in a reinstatement model of drug relapse. In that model, rats are trained to self-administer drugs of abuse such as cocaine or heroin (de Wit & Stewart, 1981, 1983). That drug-seeking behavior is later extinguished. Presentation of a non-contingent sample of the drug (priming) reinstates drug seeking. That type of priming effect is commonly referred to as priming-induced reinstatement, which is the re-establishment of a learned behavior that had previously been extinguished. In contrast, the priming effect of rewards discussed here is the invigoration a well-established behavior that has not undergone extinction.

Dopamine transmission is highly implicated in reward (Edmonds & Gallistel, 1977; Franklin, 1978; Franklin & McCoy, 1979; Gallistel & Karras, 1984; White, 1989; Berridge & Robinson, 2003; Wise, 2008). For example, Franklin and McCoy (1979) showed that following pimozide administration, a D2 family receptor (D2R) antagonist, electrical intracranial self-stimulation (eICSS) declines and eventually disappears. Presentation of a Pavlovian cue paired with reward re-establishes responding for brain stimulation. This indicates that pimozide did not abolish performance capacity but instead diminished the rewarding effect of brain stimulation. In addition, photoactivation of ventral tegmental area (VTA) dopamine neurons is sufficient to promote optical intracranial self-stimulation (oICSS) (Witten *et al.*, 2011; Steinberg *et al.*, 2014).

Dopamine transmission is also implicated in the motivating effect of reward. For example, the incentive salience theory (Robinson & Berridge, 1993; Berridge & Robinson, 1998) posits separate neural systems mediate *wanting* (incentive salience) and *liking* (hedonic value) of

reward, and that the dopamine system specifically mediates wanting (Wyvell & Berridge, 2000). In support of this, elevating dopamine levels enhances rats' willingness to work for rewarding brain stimulation at a high opportunity cost, which is the required work time to earn a reward (Hernandez *et al.*, 2010, 2012). In contrast, D2R antagonism diminishes willingness to work for rewarding brain stimulation at high opportunity cost (Trujillo-Pisanty *et al.*, 2014).

Based on those studies, it is expected that the priming effect of electrical brain stimulation is also mediated by dopamine transmission. On the contrary, the priming effect of electrical brain stimulation persists following D2R antagonism with pimozide (Wasserman *et al.*, 1982; Chapter 2). However, pimozide also has affinity for serotonin $5HT_7$ receptors. Thus, the involvement of dopamine transmission in the priming effect of rewards cannot be ruled out based on those studies.

In the present study, we studied whether dopamine transmission is necessary for the priming effect of electrical brain stimulation using a more selective D2R antagonist. In experiment 1, the behavioral design previously used in Chapter 2 was modified to become more analogous to the runway paradigm used by Gallistel and colleagues (Reid *et al.*, 1973; Edmonds & Gallistel, 1974; Gallistel *et al.*, 1974; Stellar & Gallistel, 1975; Wasserman *et al.*, 1982). This was done in the hope of eliminating a discrepancy between the results reported in Chapter 2 and those reported by Reid *et al.* (1973). Whereas a priming effect was seen in every rat tested by Reid *et al.* (1973), we observed them in 25% to 75% of our rats in Chapter 2. In experiment 2, we examined whether administration of a selective D2R antagonist, eticlopride, would block the priming effect. This drug is a more selective D2R antagonist (Hall *et al.*, 1985; Martelle & Nader, 2008) than pimozide, the drug employed by Wasserman *et al.* (1982) and in Chapter 2. We predicted that modifications to our method would improve the incidence of a priming effect of electrical brain stimulation, and that this priming effect would persist following D2R antagonism.

2. Method

2.1 Subjects

Male Long-Evans rats (bred at Concordia University, n = 8) were pair-housed in Plexiglas® cages (46 cm length x 26 cm width x 21 cm height) located in a vivarium with a reversed 12-hour light-dark cycle (lights off from 0800 to 2000 h). Throughout the study, rats had *ad libitum* access to food and water. A mix of Teklad corncob and Sani-Chips® (Envigo,

Madison, Wisconsin, USA) was used as bedding and cages were enriched with shredded paper and a tunnel toy. After the rats received bilateral electrode implantations, they were housed individually for the remainder of the experiment. Behavioral tests were conducted during the dark phase of the diurnal cycle. The protocols used were in accordance with guidelines established by Concordia University's Animal Research Ethics Committee's Terms of Reference and the Canadian Council on Animal Care's Guide to the Care and Use of Experimental Animals.

2.2 Electrode Implantation

Each rat weighed at least 350 g at the time of surgery. Ketamine-xylazine (10 mg/kg, Bioniche, Belleville, ON, Canada; Bayer Healthcare, Toronto, ON, Canada) was administered intraperitoneally (i.p) to induce anesthesia. This was followed by a subcutaneous (s.c.) injection of atropine sulfate (0.05 mg/kg, Sandoz, Boucherville, QC, Canada) to reduce bronchial secretions and penicillin (0.3 ml, s.c., Vetoquinol, Lavaltrie, QC, Canada) to prevent infections. Xylocaine jelly (AstraZeneca, Mississauga, ON, Canada) was applied to the external auditory meatus to diminish discomfort due to the stereotaxic ear bars. After placing the rat in the stereotaxic frame, a mixture of isoflurane and oxygen (Pharmaceutical Partners of Canada Inc., Richmond Hill, ON, Canada) was delivered through a snout mask to maintain anesthesia. Four to six burr holes were drilled into the skull and stainless-steel screws were threaded. The copper wire end of the current return (anode) was wrapped around two screws, and the opposite end had a gold-plated Amphenol connector. Monopolar stainless-steel electrodes were custom-made from insect pins (size: 000) insulated with Formvar enamel, leaving 0.5 mm of tip bare. The free end of the current-return wire was wrapped around two skull screws (which served as the anode), and the opposite end was terminated in a gold-plated Amphenol connector. Electrodes were bilaterally aimed at the lateral hypothalamic level (LH, AP: -2.8 from bregma, ML: ±1.7, DV: -8.8-9.0 from skull surface) of the medial forebrain bundle (MFB) and secured to the skull with dental acrylic. The Amphenol connectors were inserted into a McIntyre miniature connector (Scientific Technology Centre, Carleton University, Ottawa, ON, Canada) that was attached to the skull and skull-screw anchors using dental acrylic. The rats were allowed at least one week to recover from the surgery before self-stimulation training commenced. See Figure 1 for electrode placements.

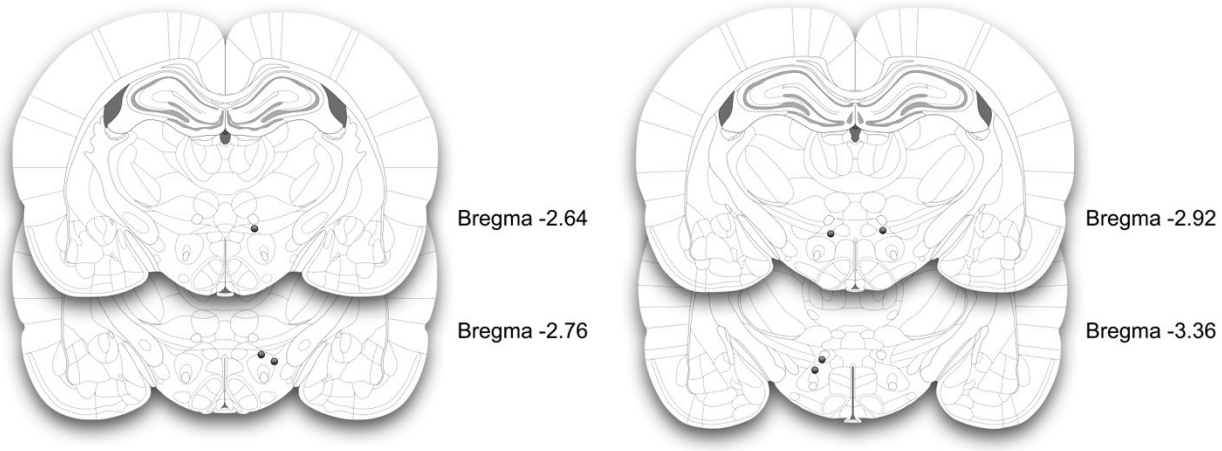

Figure 1. Placement of electrode tips. Each electrode tip was located within the boundary of the LH level of the MFB, as determined by the Paxinos and Watson (2007) atlas. Due to issues with tissue collection, the electrode placement for rat 47 is missing.

2. 3 Apparatus

The operant chambers (34 cm long x 24 cm wide x 66 cm high) were composed of wire-mesh floors (8 cm above the base), a transparent Plexiglas® front panel, an amber house light (10 cm above the mesh floor), and two retractable levers (ENV-112B, MED Associates, St. Albans, Vermont, USA). A lever was located on the left and right sides of the box and a cue light (1 cm) positioned 4 cm above each lever. An electrical swivel centered at the top of the box allowed animals to move freely with the stimulation leads.

The temporal parameters of the electrical stimulation and pulse amplitude were determined by a computer-controlled digital pulse generator and constant-current amplifier, respectively. Experiments were controlled by, and data were collected with, a custom-written computer program ("PREF3", Steve Cabilio, Concordia University, Montreal, QC, Canada).

Training. Rats were each screened to determine which electrode (left or right hemisphere) and which electrical current promoted vigorous lever pressing with minimal to no motor effects. The rats responded to currents between 210 to 440 microamperes (μA). This is a common range of current that promotes eICSS of the LH (Hernandez *et al.*, 2007; Solomon *et al.*, 2015). The pulse frequency of the priming and reward stimulation ranged from 184 pulses per second (pps) to 242 pps. The settings determined for each rat were used throughout the subsequent experiments.

Rats were trained to press a single lever for electrical brain stimulation on a fixed-ratio eight (FR8) schedule. The reward stimulation consisted of a single 0.5-second (s) train of 0.1-millisecond (ms) cathodal pulses. Once the operant behavior was stable, rats were trained in the testing paradigm. Rats learned to press a setup that did not deliver reward but activated the extension of a reward lever that was located on the opposite wall of the chamber and armed on an FR1 schedule. Initially, the setup lever was armed on an FR1 schedule; the response requirement was then increased gradually to FR8.

As in our previous method for measuring the priming effect of electrical brain stimulation (Chapter 2), the purpose of the setup lever was for the rat to be located in the same position at the start of each trial and to prevent superstitious behaviors. However, in the previous studies, the response requirement on the setup lever was only one press followed by a variable number of presses on the reward lever to earn brain stimulation. In the present study, rats pressed the setup

lever eight times, which activated the extension of the reward lever. A single response on the reward lever delivered a single train of brain stimulation.

Testing. Rats initially underwent a warm-up session during which they pressed a single lever for electrical brain stimulation. The test session commenced following the warm-up. As illustrated in Figure 2b, before a trial started there was a 30-s inter-trial interval (ITI) that consisted of a waiting period, a pretrial cues period, and a delay. The first component of the ITI was a waiting period, during which the house light remained off for 20 s. The waiting period was followed by delivery of pretrial cues that signaled the start of a trial. Cues included flashing of the amber house light (10 cycles of 0.5 s on, 0.5 s off) and delivery of priming stimulation. On primed trials, the reward lever extended and was armed on an FR1 schedule. The reward lever remained extended for 20 s, giving the rats the opportunity to press for 10 separate priming stimulations that consisted of 0.5-s trains of 0.1ms cathodal pulses. After completing each FR1 response requirement, there was a 1-s timeout period during which the priming stimulation was delivered and the reward lever retracted. The 20-s timer was paused during this timeout period. After the last priming stimulation was obtained, there was a 5-s delay. On non-primed trials, the reward lever did not extend at the end of the 10-s flashing period of the amber house light. Instead, the end of the ITI was followed by the post-priming delay. During the delay, the house light returned to its off state and the reward lever remained retracted. At the end of the delay, a trial began with the extension of the setup lever and its cue light. The rat was required to press the setup lever eight times (FR8) to activate the extension of the reward lever, which was armed on a FR1 schedule. Upon completion of the response requirement and receipt of the reward stimulation, a new ITI began. If the setup lever was not pressed, then the reward lever did not extend and a new ITI immediately commenced.

Each test session consisted of 42 trials. The first two trials of a session were primed trials that served as warmup trials and were excluded from analyses. Subsequent trials consisted of four cycles of five primed and five non-primed trials (Figure 2c). Each test session lasted approximately 35 minutes. Data from each rat in each experimental condition were collected from at least 10 test sessions, unless otherwise stated.

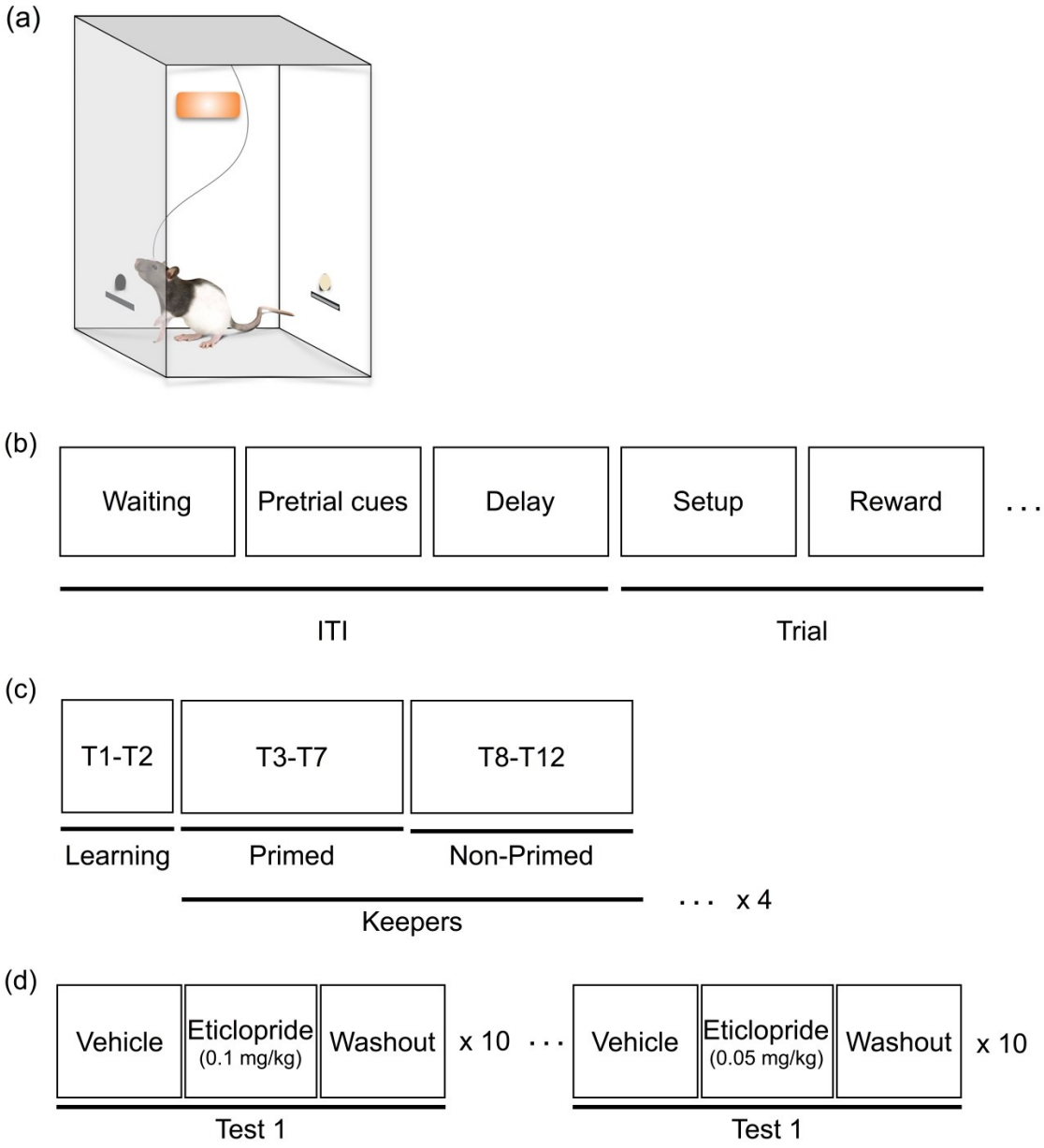

Figure 2. An image of the operant-conditioning chamber and schematics of test procedures. (a) The operant-conditioning chamber contains a setup lever (left) and a reward lever (right). (b) Preceding a trial was an ITI, which started with a waiting period. This was followed by a pretrial cues period, during which cues were delivered to signal that the start of a trial was approaching. On primed trials, the reward lever extended to allow rats to self-administer priming stimulations. The pretrial cues period was followed by a delay. A trial commenced with the extension of the setup lever. Eight presses (FR8) on the setup lever activated the extension of the reward lever located at the opposite side of the chamber. A single press on the reward lever delivered

rewarding brain stimulation and initiated the start of a new ITI. The duration of each event within an ITI or trial is mentioned in the method section. (c) The first two trials (T1-T2) in a test were primed trials that served as “learning” trials, which were excluded from data analyses. Subsequent “keeper trials” (T3-T42) were analyzed. The keeper trials consisted of a set of five primed trials and five non-primed trials, which were repeated in four cycles to reach a total to 40 keeper trials. (d) Tests with drug were conducted in three-day cycles consisting of a vehicle day, drug day, and washout day. A single dose of eticlopride was administered in at least 10 separate tests, unless otherwise stated.

2.4 Experiment 1

We did not consistently observe a priming effect of electrical brain stimulation in Chapter 2. In contrast, Reid *et al.* (1973) reported that the priming effect of electrical brain stimulation was observed in 100% of the rats tested when measured using a runway paradigm. In the hope of obtaining a more consistent priming effect, we modified the operant paradigm used in Chapter 2 to become more analogous to the runway paradigm used by Gallistel and colleagues (Reid *et al.*, 1973; Edmonds & Gallistel, 1974; Gallistel *et al.*, 1974; Stellar & Gallistel, 1975).

A chained schedule was in effect, requiring rats to press a setup lever eight times to gain access to a reward lever. The work performed in meeting the response requirement on the setup lever could be considered analogous to the work performed in traversing the runway. Once the rat completed the response requirement on the setup lever the reward lever became available. This is comparable to the rat reaching the goal box located at the end of the alley. When rats reached the goal box, they pressed the lever in the goal box once (FR1) to obtain a single train of brain stimulation. Likewise, in the present study, once the reward lever became available, a single press on the reward lever delivered electrical brain stimulation.

An additional change implemented in the present study was the mode of delivering the priming stimulations. In chapter 2, priming stimulations were automatically delivered to the rats. These high frequency priming stimulations could have produced aversive effects that affected the incidence of a priming effect. Here, rats self-administered the priming stimulations. Allowing the rats to control the pace of receiving the priming stimulations was thought to reduce the aversive effects of high frequency priming stimulations. Gallistel *et al.* (1974) showed that self-administered priming stimulations can produce a priming effect of electrical brain stimulation. All in all, these modifications to the operant design were hypothesized to improve the incidence of a priming effect.

2.5 Experiment 2

To examine the role of dopamine transmission in the priming effect of electrical brain stimulation, eticlopride, a D2R antagonist, was administered. Eticlopride (0.1 or 0.05 mg/kg, Sigma, St. Louis, MO) was dissolved in physiological saline (0.9%). These doses were based on data from a previous pilot study (data not shown) and Lazenka *et al.* (2016). Vehicle (0.9% physiological saline) or one of the two doses of eticlopride was administered intraperitoneally (i.p) 30 minutes prior to testing. Tests were conducted in three-day cycles consisting of a vehicle day, drug day, and washout day (Figure 2d). Tests were first conducted at the highest dose of eticlopride (0.1 mg/kg) followed by the lower dose (0.05 mg/kg). We aimed to collect 10 vehicle-drug cycles for each dose of eticlopride, unless otherwise stated, before collecting data for the next lower dose.

2.6 Statistical Analyses

For each rat, data were analyzed for each priming condition (high or no priming) and drug dose (0, 0.1, or 0.5 mg/kg). The mean and median were calculated for both initial speed and reward rate measures. The medians were used to calculate effect sizes and difference ratios. Data were analyzed and graphs were plotted using custom-written MatLab scripts (The MathWorks, Natick, MA).

Initial Speed. Initial speed is the inverse of the latency (s) to press the setup lever following lever extension. A larger initial speed number indicates that a rat was quicker to initiate the first press on the setup lever. This is akin to how fast a rat exited the start box to traverse the alley in the runway paradigm.

Reward Rate. Reward rate is the inverse of the total time (s) elapsed between the start of the trial and the delivery of the reward. Higher reward rates reflect more vigorous reward pursuit. This transformation is analogous to the running speed measure reported in Gallistel's investigations on the priming effect of electrical brain stimulation (Gallistel *et al.*, 1974; Stellar & Gallistel, 1975; Wasserman *et al.*, 1982).

Confidence Intervals. Bootstrapping (Efron & Tibshirani, 1986) was used to determine mean and median speeds and their surrounding confidence intervals (CI). Data from each priming condition were randomly sampled with replacement to generate 1000 resampled datasets. The upper and lower 2.5% of the distribution were defined as the bounds of a 95% CI.

Distributions. The distribution of the data was visualized with violin plots based on kernel-density estimation (KDE). This is a non-parametric method for estimating the probability-density function of a random variable, such as response speed. In contrast to traditional statistics, KDE addresses the data smoothing problem without prior parametric assumptions (e.g., normality). Instead, KDE creates smooth distributions based on a given sample of data.

Cliff's Delta. Visual inspection shows that the speed measures are both skewed and bimodal. Thus, a non-parametric analysis of effect size was employed. Cliff's delta is an effect size measure used for ordinal data that does not require assumptions about the distribution of the data. A Cliff's delta value near one indicates that high priming produced reliably faster response speeds compared to no priming. No difference between the high- and no-priming medians would yield a Cliff's delta value of zero. Bootstrapping (Efron & Tibshirani, 1986) with replacement was used to calculate both Cliff's delta and its surrounding 95% CI.

Difference ratio. A statistic was developed to assess the magnitude of the difference between speed measures obtained in the high- and no-priming conditions. The ratio of the difference between the two group medians was first calculated by means of, bootstrapping with replacement (1000 resampled medians for both the high and no priming conditions). The difference between the resampled medians was then normalized by the resampled grand median to yield the median difference ratio.

Criteria for a priming effect. A two-criterion approach was used to determine whether the results demonstrate a reliable and meaningful behavioral difference between the high- and no-priming conditions. The first criterion was a Cliff's delta greater than zero surrounded by a 95% CI excluding zero. The second criterion was a median difference ratio equal to or greater than .10 surrounded by a 95% CI excluding zero. A difference that met the criterion for Cliff's delta but not the median-difference-ratio criterion was considered statistically reliable but too small to be regarded as meaningful.

3. Results

3.1 Experiment 1: Self-Administered Priming Stimulation

Based on the two-criterion approach, we showed a reliable and meaningful priming effect of electrical brain stimulation with our modified method. Following high priming, seven out of eight rats showed a 25% to 98% increase in initial speed relative to the grand median (Figure 4a

& 4c; Table 1). All eight rats showed a 13% to 43% increase in reward rate relative to the grand median in response to high priming (Figure 4b & 4d; Table 2).

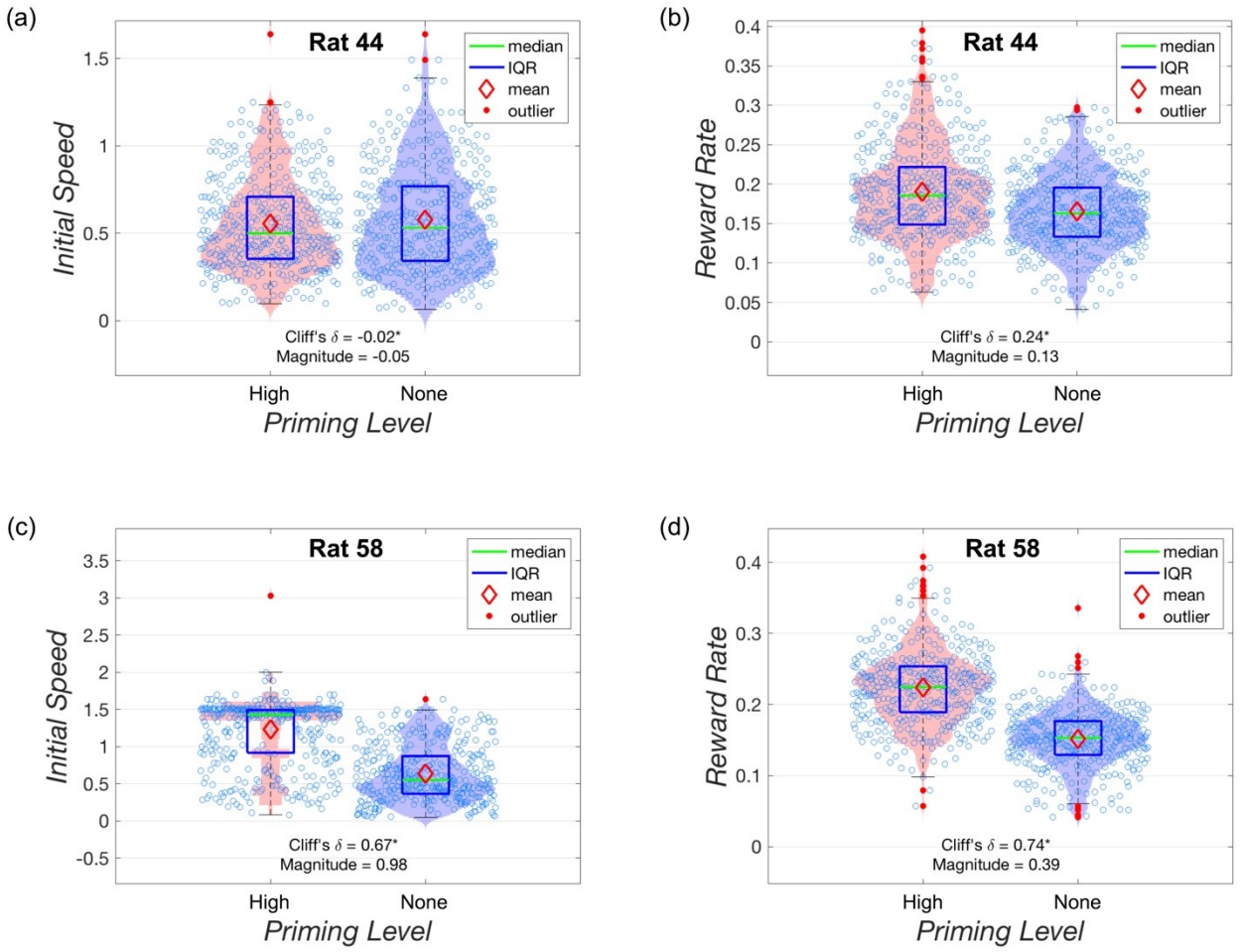

Figure 3. The priming effect is present when rats self-administer the priming stimulations. These are representative data from rats 44 and 58. Rat 44 shows (a) no priming effect on the initial speed measure but (b) does show a meaningful and reliable priming effect on the reward rate measure. (c & d) Rat 58 shows a reliable and meaningful priming effect on both speed measures. Blue open-dots represent individual data points, red-filled dots represent outliers, the green line represents the median, the red diamond represents the mean, and blue boxes represent the interquartile range.

Table 1

Initial Speed following Self-Administered Priming Stimulation

Rat No.	Median			Median Difference		Cliff's Delta	
	HP	NP	Grand	Ratio	95% CI	δ	95% CI
44	0.50	0.53	0.52	-0.05*	[-0.15, 0.06]*	-0.02*	[-0.09, 0.06]*
45	0.98	0.75	0.93	0.25	[0.12, 0.30]	.034	[0.27, .041]
46	1.56	0.81	1.14	0.65	[0.60, 0.72]	0.81	[0.77, 0.85]
47	1.61	0.65	1.10	0.88	[0.80, 0.97]	.085	[0.80, 0.88]
48	1.25	0.62	0.90	0.70	[0.65, 0.76]	.90	[0.87, 0.93]
50	1.92	1.30	1.56	0.40	[0.32, 0.47]	0.71	[0.66, 0.76]
55	1.52	0.93	1.25	0.48	[0.44, 0.52]	0.87	[0.83, 0.91]
58	1.43	0.55	0.90	0.98	[.89, 1.09]	0.67	[0.60, 0.72]

* Did not meet the criteria for a reliable and/or meaningful priming effect.

Notes. HP = high priming. NP = no priming. Grey highlight emphasizes which rats did not show a reliable and/or meaningful priming effect.

Table 2

Reward Rate following Self-Administered Priming Stimulation

Rat No.	Median			Median Difference		Cliff's Delta	
	HP	NP	Grand	Ratio	95% CI	δ	95% CI
44	0.19	0.16	0.17	0.13	[0.09, 0.17]	0.24	[0.17, 0.31]
45	0.28	0.23	0.25	0.20	[0.18, 0.23]	0.51	[0.44, 0.57]
46	0.34	0.22	0.26	0.43	[0.39, 0.45]	0.89	[0.86, 0.91]
47	0.31	0.21	0.25	0.39	[0.36, 0.41]	0.81	[0.77, 0.85]
48	0.25	0.19	0.22	0.27	[0.24, 0.30]	0.66	[0.61, 0.70]
50	0.35	0.31	0.32	0.13	[0.11, 0.15]	0.54	[0.47, 0.60]
55	0.30	0.23	0.26	0.28	[0.25, 0.31]	0.67	[0.60, 0.73]
58	0.22	0.15	0.18	0.39	[0.36, 0.42]	0.74	[0.69, 0.79]

Notes. HP = high priming. NP = no priming. Grey highlight emphasizes which rats did not show a reliable and/or meaningful priming effect.

3.2 Experiment 2: Eticlopride & The Priming Effect of Electrical Brain Stimulation

To examine if the priming effect of electrical brain stimulation depends on D2R signaling, rats were tested with eticlopride (0.1mg/kg, n = 6; 0.05 mg/kg, n = 3). Of the six rats tested with 0.1 mg/kg of eticlopride, all six rats showed a reliable and meaningful priming effect in response to vehicle on the initial speed measure. Five rats showed a reliable and meaningful priming effect in response to vehicle on the reward rate measure. Relative to the grand median, high priming increased initial speed by 24% to 89% (Figure 5a; Table 3) and it increased reward rate by 23% to 93% (Figure 5b; Table 3). In response to 0.1 mg/kg of eticlopride, two rats showed a reliable and meaningful priming effect in the initial speed and reward rate measures. They showed a 58% to 60% increase in initial speed relative to the grand median following high priming (Figure 5c; Table 4) and a 34% to 43% increase in reward rate relative to the grand median following high priming (Figure 5d; Table 4).

All three rats tested with 0.05 mg/kg of eticlopride showed a reliable and meaningful priming effect in the initial speed measure in response to vehicle and eticlopride. Relative to the grand median, high priming increased initial speed by 15% to 89% following vehicle and 15% to 55% following 0.05 mg/kg of eticlopride (Figure 6a & 6c; Table 5). Two out of the three rats showed a reliable and meaningful priming effect in the reward rate measure in response to vehicle and eticlopride. Relative to the grand median, high priming increased the reward rate by 22% to 28% in response to vehicle and 26% to 28% in response to eticlopride (Figure 6b & 6d; Table 6).

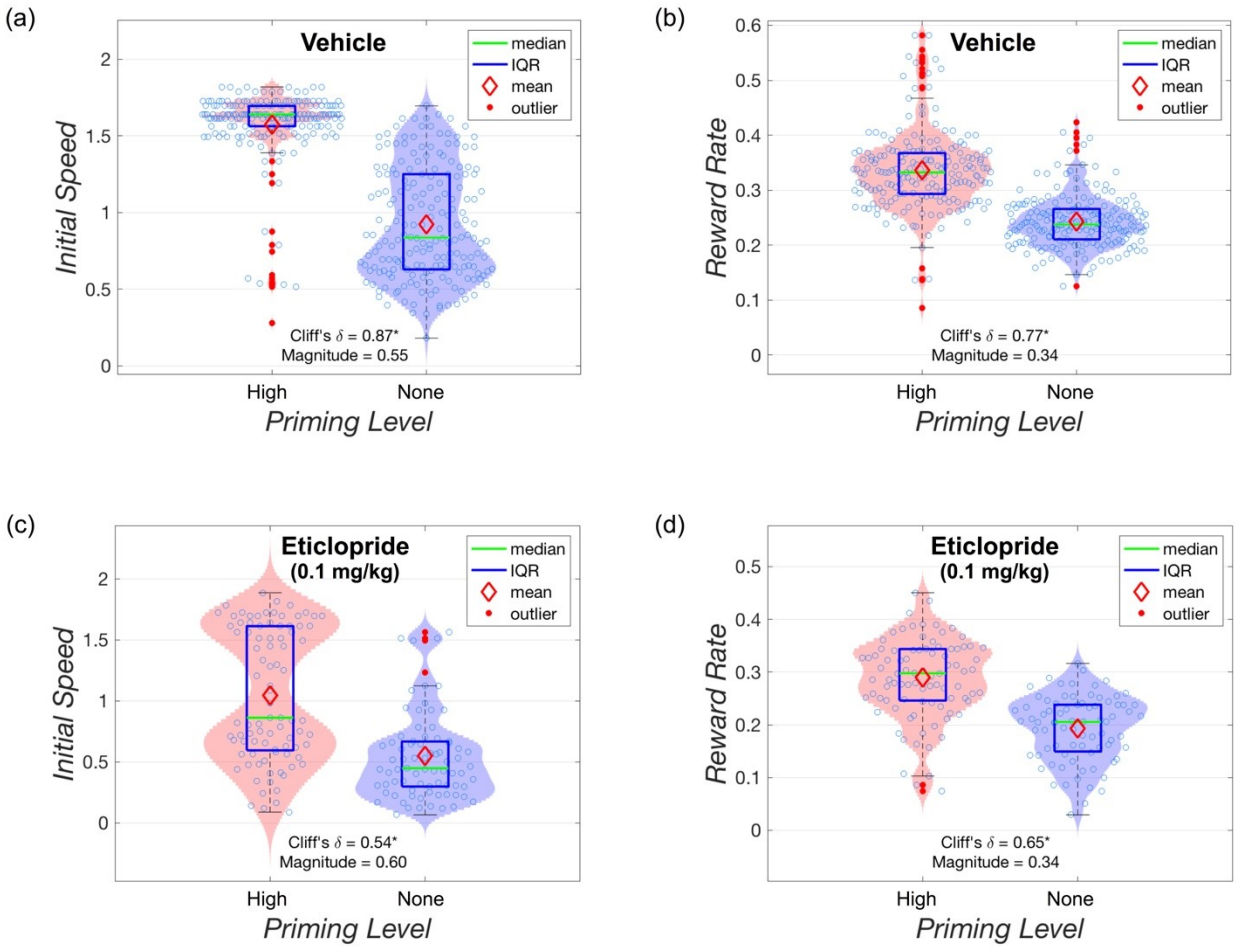

Figure 4. Two of out six rats show a priming effect following both vehicle and 0.1 mg/kg of eticlopride. These are representative data from rat 47. (a) Initial speed and (b) reward rate in response to vehicle. (a) Initial speed and (b) reward rate in response to 0.1 mg/kg of eticlopride. Blue open-dots represent individual data points, red-filled dots represent outliers, the green lines represent medians, red diamonds represent means, and blue boxes represent interquartile ranges.

Table 3

Initial Speed following Self-Administered Priming Stimulation: Vehicle vs. 0.1 mg/kg Eticlopride

	Vehicle							Eticlopride (0.1 mg/kg)						
Rat No.	Median			Median Difference		Cliff's Delta		Median			Median Difference		Cliff's Delta	
	HP	NP	Grand	Ratio	95% CI	δ	95% CI	HP	NP	Grand	Ratio	95% CI	δ	95% CI
46[a]	1.56	1.15	1.41	0.31	[0.21, 0.39]	0.89	[0.79, 0.97]	1.43	0.82	1.13	0.58	[0.39, 0.94]	0.70	[0.46, 0.90]
47	1.64	0.83	1.45	0.55	[0.47, 0.61]	0.87	[0.81, 0.92]	0.85	0.45	0.65	0.60	[0.36, 1.23]	0.54	[0.42, .064]
48	1.16	0.89	1.05	0.25	[0.18, 0.33]	0.41	[0.29, 0.53]	0.92	0.78	0.85	0.14	[-0.05, 0.32]*	0.23	[0.05, 0.38]
50[a]	2.94	1.38	1.87	0.84	[0.50, 1.32]	0.81	[0.65, 0.92]	1.25	1.09	1.11	0.14	[-0.53, 0.80]*	0.21	[0.00, 0.40]*
55	1.29	0.69	0.84	0.70	[0.54, 0.81]	0.59	[0.50, 0.68]	0.58	0.57	0.58	0.02*	[-0.10, 0.15]*	0.08	[0.00, .018]*
58	1.39	1.06	1.27	0.24	[0.14, 0.30]	0.24	[0.13, 0.33]	0.63	0.64	0.64	-0.09*	[-0.71, 0.54]*	0.00	[-0.14, 0.15]*

[a] Data are from two test sessions only.

* Did not meet the criteria for a reliable and/or meaningful priming effect.

Notes. HP = high priming. NP = no priming. Grey highlight emphasizes which rats did not show a reliable and/or meaningful priming effect.

Table 4

Reward Rate following Self-Administered Priming Stimulation: Vehicle vs. 0.1 mg/kg Eticlopride

	Vehicle							Eticlopride (0.1 mg/kg)						
Rat No.	Median			Median Difference		Cliff's Delta		Median			Median Difference		Cliff's Delta	
	HP	NP	Grand	Ratio	95% CI	δ	95% CI	HP	NP	Grand	Ratio	95% CI	δ	95% CI
46[a]	0.33	0.23	0.27	0.34	[0.26, 0.42]	0.93	[0.83, 0.99]	0.33	0.21	0.27	0.43	[0.34, 0.51]	0.90	[0.68, 1.00]
47	0.33	0.24	0.28	0.34	[0.30, 0.38]	0.77	[0.70, 0.84]	0.29	0.21	0.24	0.34	[0.24, 0.43]	0.65	[0.55, 0.76]
48	0.78	0.24	0.22	0.09*	[0.05, 0.13]	0.23	[0.12, 0.34]							
50[a]	0.39	0.32	0.34	0.19	[0.13, 0.24]	0.64	[0.43, 0.82]	0.32	0.28	0.30	0.13	[-0.01, 0.59]*	0.32	[0.16, 0.47]
55	0.25	0.19	0.21	0.29	[0.24, 0.34]	0.59	[0.50, 0.67]	0.20	0.18	0.19	0.09*	[0.02, 0.17]	0.22	[0.11, 0.32]
58	0.22	0.19	0.21	0.16	[0.10, 0.20]	0.26	[0.16, 0.36]	0.19	0.19	0.19	-0.02*	[-0.22, 0.13]*	0.07	[-0.08, 0.21]

[a] Data are from two test sessions only.

* Did not meet the criteria for a reliable and/or meaningful priming effect.

Notes. HP = high priming. NP = no priming. Grey highlight emphasizes which rats did not show a reliable and/or meaningful priming effect.

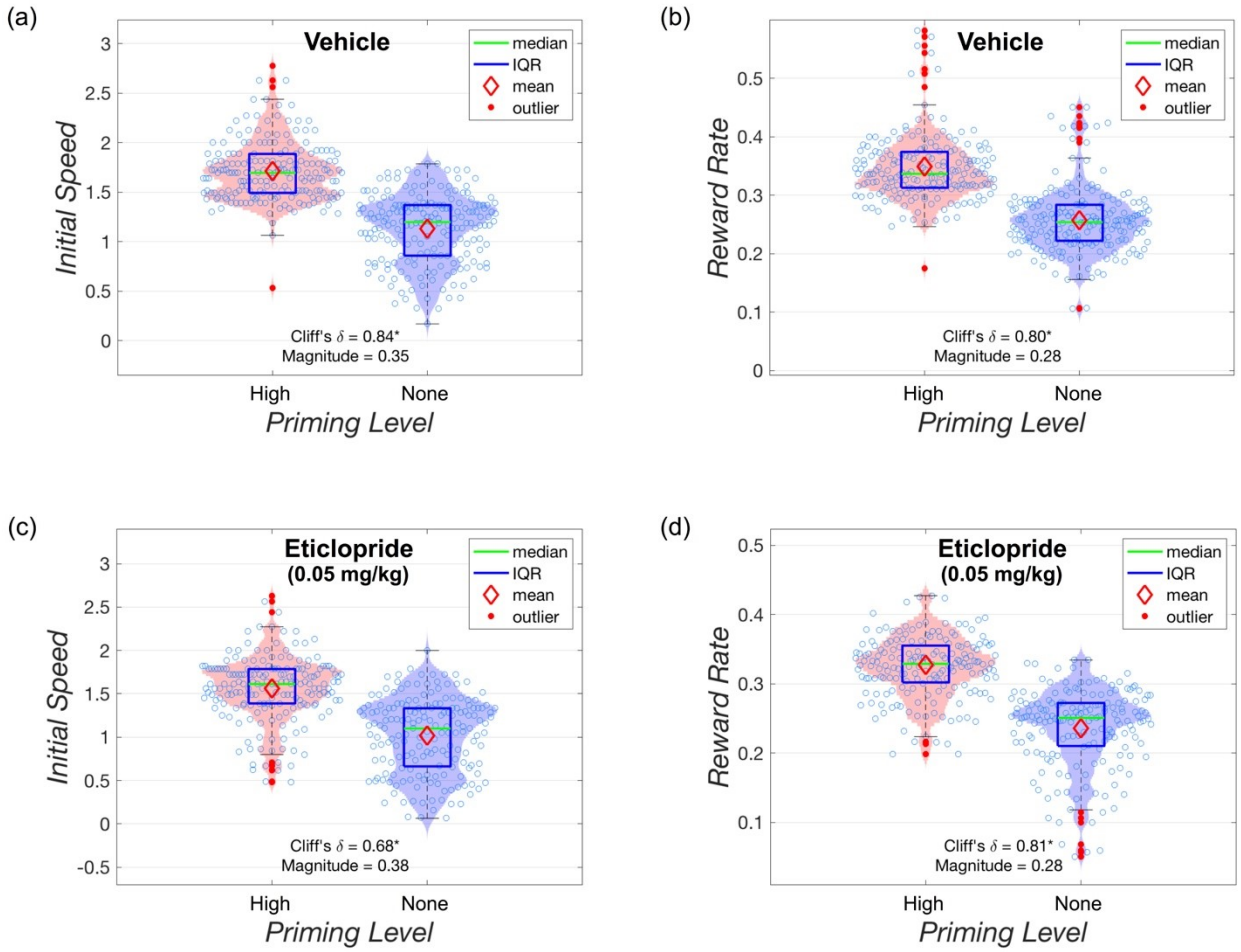

Figure 5. The priming effect persists following 0.05 mg/kg of eticlopride. These are representative data from rat 46. (a) Initial speed and (b) reward rate in response to vehicle. (a) Initial speed and (b) reward rate in response to 0.05 mg/kg of eticlopride. Blue open-dots represent individual data points, red-filled dots represent outliers, the green lines represent medians, red diamonds represent means, and blue boxes represent interquartile ranges.

Table 5

Initial Speed following Self-Administered Priming Stimulation: Vehicle vs. 0.05 mg/kg Eticlopride

	Vehicle							Eticlopride (0.05 mg/kg)						
Rat No.	Median			Median Difference		Cliff's Delta		Median			Median Difference		Cliff's Delta	
	HP	NP	Grand	Ratio	95% CI	δ	95% CI	HP	NP	Grand	Ratio	95% CI	δ	95% CI
46	1.69	1.19	1.45	0.34	[0.28, 0.39]	0.84	[0.77, 0.89]	1.61	1.10	1.37	0.38	[0.27, 0.46]	0.68	[0.59, 0.76]
48[b]	0.92	0.79	0.85	0.15	[0.01, 0.28]	0.16	[0.05, 0.27]	0.97	0.83	0.88	0.15	[0.03, 0.29]	0.19	[0.04, 0.36]
50	3.23	1.49	1.90	0.89	[0.71, 1.13]	0.80	[0.74, 0.90]	2.27	1.33	1.64	0.55	[0.45, 0.66]	0.62	[0.52, 0.70]

[b] Data are from nine test sessions only.

Notes. HP = high priming. NP = no priming. Grey highlight emphasizes which rats did not show a reliable and/or meaningful priming effect.

Table 6

Reward Rate following Self-Administered Priming Stimulation: Vehicle vs. 0.05 mg/kg Eticlopride

	Vehicle							Eticlopride (0.05 mg/kg)						
Rat No.	Median			Median Difference		Cliff's Delta		Median			Median Difference		Cliff's Delta	
	HP	NP	Grand	Ratio	95% CI	δ	95% CI	HP	NP	Grand	Ratio	95% CI	δ	95% CI
46	0.34	0.25	0.30	0.28	[0.26, .32]	0.80	[0.74, .86]	0.33	0.25	0.28	0.28	[0.25 0.31]	0.81	[0.75, 0.87]
48[b]	0.18	0.18	0.18	0.03*	[-0.02, 0.09]*	0.11	[0.00, 0.22]*							
50	0.39	0.31	0.33	0.22	[0.19, 0.26]	0.68	[0.61, 0.76]	0.38	0.29	0.33	0.25	[0.21, 0.29]	0.76	[0.69, 0.83]

[b] Data are from nine test sessions only.

* Did not meet the criteria for a reliable and/or meaningful priming effect.

Notes. HP = high priming. NP = no priming. Grey highlight emphasizes which rats did not show a reliable and/or meaningful priming effect.

4. Discussion

Electrical brain stimulation elicits a rewarding effect and a priming effect. The rewarding effect is expressed as an enhanced inclination to seek brain stimulation following receipt of an intense reward. The priming effect is thus interpreted as a transient boost in vigor to work for more brain stimulation. Dopamine transmission has been highly implicated in reward and motivation. However, results from Wasserman *et al.* (1982) and Chapter 2 of this thesis indicate that the priming effect might not depend on dopamine transmission. In accordance with those findings, we showed that the priming effect of electrical brain stimulation persists in response to a more selective D2R antagonist. Therefore, although dopamine transmission plays and important role in reward and motivation, the priming effect of electrical brain stimulation may not depend on D2R signaling.

4.1 The Priming Effect of Electrical Brain Stimulation & Dopamine Transmission

The priming effect of rewards manifests two key properties of an enhancement in motivation: it *directs* and *invigorates* goal-seeking behavior. Deutsch *et al.* (1964) showed that thirsty rats primed with electrical brain stimulation are more likely to choose brain stimulation over water in a T-maze. This shows that when faced with a competing reward, priming can *direct* preference for the primed stimulus. Gallistel *et al.* (1974) showed that following priming with electrical brain stimulation rats run faster to the end of a runway to work for rewarding brain stimulation. This increase in running speed reflects the invigorating effect of priming on reward seeking.

Dopamine transmission has been implicated in many variables that affect motivation. The incentive salience hypothesis postulates that the midbrain dopamine system mediates the incentive salience (i.e., *wanting*) of reward but not the pleasurable aspects (i.e., *liking*) of reward (Robinson & Berridge, 1993; Berridge & Robinson, 1998). In support of this idea, Wyvell and Berridge (2000) showed that microinjections of amphetamine into the nucleus accumbens (NAc) shell potentiates cue-induced operant responding for sucrose (interpreted as a change in *wanting*) but not hedonic reactions to sucrose (interpreted as a change in *liking*). Another variable that affects motivation is the cost of obtaining rewards. Dopamine depletion in the NAc attenuates rats' willingness to work for reward that requires a high effort cost (Aberman & Salamone, 1999; Salamone *et al.*, 2001). Findings from the reward-mountain model are consistent with the idea that subjective costs are modulated by tonic dopamine (Hernandez *et al.*, 2010, 2012; Trujillo-

Pisanty *et al.*, 2014). These findings demonstrate that dopamine transmission is important for motivation.

The eticlopride doses employed here have previously been shown to reduce the rewarding effect, but not the priming effect, of brain stimulation and food. Lazenka *et al.* (2016) showed that eticlopride doses between 0.032 mg/kg and 0.1 mg/kg attenuate eICSS. In Chapter 4, Evangelista *et al.* (2019) showed that 0.05 mg/kg of eticlopride diminishes responding for food. In a pilot study, we showed 0.1 mg/kg of eticlopride completely eliminates responding for food. The 0.1 mg/kg dose employed here may have been too high for most of the rats we tested. This may explain why the priming effect disappeared in the majority of the rats that received the higher dose of eticlopride (Tables 3 & 4).

We showed that the priming effect persists following a lower, yet still behaviorally effective, dose of eticlopride (Tables 5 & 6). This is consistent with Wasserman *et al.'s* (1982) results that showed primed rats continue to run faster to the goal box to earn rewarding brain stimulation following D2R antagonism with pimozide. They observed that rats elicit a priming effect in the first few trials and then cease to perform altogether. Since the rewarding effect of brain stimulation is sensitive to dopamine receptor antagonism (Gallistel *et al.*, 1982), pimozide diminishes performance across the session. Nevertheless, they showed a priming effect is present prior to pimozide blocking the rewarding effect of electrical brain stimulation.

4.2 Improvements to Measuring the Priming Effect of Electrical Brain Stimulation

In Chapter 2, a new method was designed to measure the priming effect of electrical brain stimulation based on rates of lever pressing. A priming effect was observed in some, but not all, rats. One possible explanation for the inconsistent incidence of a priming effect is that the design used in Chapter 2 is not as analogous to the runway as we had thought.

In the runway paradigm, rats are placed in a start box and the alley is blocked by a start door. A trial starts when the start door opens, and the rat travels to the goal box located at the end of the alley to lever press for brain stimulation. In the present study, the work performed in meeting the response requirement on the setup lever was thought to be analogous to the work performed in traversing the runway. After the rat completed the response requirement on the setup lever, a 180-degree turn was required to press the reward lever once (FR1) to earn rewarding brain stimulation. This is comparable to the rat reaching the goal box at the end of the

alley and pressing a lever there to obtain a reward. The chained schedule we employed was thought to be more analogous to the runway paradigm used by Gallistel *et al.* (1974).

Another possible explanation for the inconsistent incidence of a priming effect of electrical brain stimulation seen in Chapter 2 is because of the manner in which the priming stimulations were received by the rats. In Chapter 2, priming stimulations were automatically delivered to the rats for free. However, it has been shown that rats prefer to work for a reward instead of receiving it for free (Jensen, 1963; Osborne, 1977; Inglis *et al.*, 1997). Based on this, we thought that the incidence of a priming effect of electrical brain stimulation may be more likely if we allowed the rats to earn the priming stimulations instead of receiving them for free.

Although the priming effect of electrical brain stimulation has largely been studied by delivering free samples of a reward (Edmonds & Gallistel, 1974; Gallistel *et al.*, 1974; Stellar & Gallistel, 1975; Wasserman *et al.*, 1982), it is also present when rewards are earned. Gallistel (1966) trained rats to traverse an alley to lever press for brain stimulation with no priming stimulation delivered in the start box. The ITI ranged from five s to 60 s. The response-contingent goal-box stimulation transiently energized reward seeking in a subsequent trial when the ITI was short. In another study, Gallistel *et al.* (1974) trained rats to lever press for priming stimulation in the start box. Those rats ran faster to the goal box following receipt of response-contingent priming stimulation earned in the start box. These studies showed that a priming effect of electrical brain stimulation can also be observed when rewards are earned, not free.

After modifying our method to become more analogous to a runway and to allow rats to earn the priming stimulations, we observed a more consistent priming effect of electrical brain stimulation. In Chapter 2, a priming effect of electrical brain stimulation was observed in 25% to 75% of the rats tested. Here, 88% of the rats were faster to start working for reward and 100% of the rats earned the reward faster (Tables 1 & 2).

5. Conclusion

The present study shows that a more consistent priming effect of electrical brain stimulation can be observed when the behavioral paradigm is analogous to a runway. Furthermore, we obtained evidence consistent with the notion that the priming effect of electrical brain stimulation does not depend on D2R signaling. It is widely recognized that dopamine transmission plays an important role in reward and motivation. Nevertheless, evidence from the

present study indicates that it may not be necessary for certain aspects of motivation such as the priming effect.

Chapter 4: The Priming Effect of Food Persists Following Blockade of Dopamine Receptors

Abstract

The priming effect of rewards is a boost in the vigor of reward seeking resulting from the previous receipt of a reward. Extensive work has been carried out on the priming effect of electrical brain stimulation, but much less research exists on the priming effect of natural rewards, such as food. While both reinforcement and motivation are linked with dopamine transmission in the brain, the priming effect of rewards does not appear to be dopamine-dependent. In the present study, an operant method was developed to measure the priming effect of food and then applied to investigate whether it is affected by dopamine receptor antagonism. Long-Evans rats were administered saline or one of the three doses (0.01, 0.05, 0.075 mg/kg) of the dopamine D1 receptor family antagonist, SCH23390, or the dopamine D2 receptor family antagonist, eticlopride. Although dopamine receptor antagonism affected pursuit of food, it did not eliminate the priming effect. These data suggest that despite the involvement of dopamine transmission in reinforcement and motivation, the priming effect of food does not depend on dopamine transmission.

1. Introduction

Over a half century of research implicates dopamine transmission in reinforcement and motivation. This work has been so fruitful as to risk obscuring complementary roles played by other neurotransmitter systems and other neural pathways. Research on the priming effect of electrical brain stimulation provides one line of evidence that dopamine transmission may not be essential to certain aspects of motivation. The priming effect of rewards is a transient increase in the motivation to pursue a reward after having previously received that reward (Gallistel, 1966; Reid *et al.*, 1973; Edmonds & Gallistel, 1974; Gallistel *et al.*, 1974). The finding that the priming effect of electrical brain stimulation withstands blockade of dopamine D2-like receptors (Wasserman *et al.*, 1982) is inconsistent with ideas such as the incentive salience hypothesis that links increased *wanting* in the wake of recent exposure to rewards to dopamine signaling (Robinson & Berridge, 1993; Berridge & Robinson, 1998). The neurobiological bases of the priming effect, specifically the role of dopamine transmission, remain undetermined.

It is important to distinguish the priming effect examined in this study from other effects bearing the same moniker. First, there is an identically named priming effect in a reinstatement model of drug relapse. In that model, rats are trained to self-administer drugs of abuse such as cocaine and heroin (de Wit & Stewart, 1981, 1983). Following this, the rats are forced into a period of abstinence when operant responding no longer delivers the drug. After drug-seeking behavior is extinguished, the presentation of a non-contingent sample of the drug (priming) reinstates the drug-seeking behavior. This type of priming effect is commonly referred to as priming-induced reinstatement, which is the re-establishment of a learned behavior that had previously been extinguished. This implies that the results of the original learning have not been erased but instead have been counteracted by subsequent extinction training. In contrast, the priming effect observed with electrical brain stimulation is the invigoration of a goal-directed behavior that has not undergone extinction. To differentiate these two effects, we refer to the priming-induced invigoration of a well-established behavior as the priming effect of rewards (e.g., priming effect of electrical brain stimulation or priming effect of food).

Another identically named priming effect is an increase in drive to pursue a reward following long delays, ranging from 5 minutes to 24 hours, from receiving a prime of that reward (Morgan & Fields, 1938; Liu *et al.*, 2016). In contrast, the priming effect of rewards is transient, lasting only several seconds. Deutsch *et al.* (1964) and Gallistel (1966) showed that the priming

effect of electrical brain stimulation largely dissipates within 20 seconds of the last priming stimulation. The long-lasting priming effect, which may be considered a pre-exposure effect, is not comparable to the transient priming effect of rewards discussed in this paper. Due to the enduring nature of the effect of pre-exposure, it may tap into a different motivational system and would seem to depend on memory. In contrast, there is evidence that the priming effect of electrical brain stimulation does not act on a memory-based system (Gallistel *et al.*, 1974). Thus, the priming effect of rewards and the effect of pre-exposure are considered different phenomena.

Two distinct effects of electrical brain stimulation are measured in a runway paradigm employed by Wasserman *et al.* (1982): a priming effect and a reinforcing effect. Rats were primed with non-contingent brain stimulation in a start box. Following a delay, the start door opened to give the rats access to an alley. The end of the alley contained a goal lever that delivered electrical brain stimulation when pressed. The priming effect of electrical brain stimulation received in the start box is expressed as a transient increase in the speed with which the rat traverses the alley and presses the goal box lever. The reinforcing effect of the response-contingent brain stimulation received in the goal box is expressed as the proclivity of the rat to run down the alley and the value it assigns to the stimulation available there. Gallistel *et al.* (1974) showed that a change in the strength of response-contingent goal-box stimulation leads to gradual adjustments of performance over multiple trials until the rat learns the updated value of the stimulation and a new, stable performance level is attained. In contrast, they also demonstrated that performance adjusts immediately following a change in the strength of the non-contingent start-box stimulation. These results indicate that the priming and reinforcing effects of electrical brain stimulation are independent.

The priming effect of electrical brain stimulation manifests two defining properties of an increase in motivation: it both *directs* and *potentiates* reward-seeking behavior. In a T-maze, Deutsch *et al.* (1964) offered thirsty rats a choice between water and electrical brain stimulation. Rats that received no pretrial priming brain stimulation preferentially chose the arm that contained water, whereas priming with brain stimulation increased the probability of choosing electrical brain stimulation over water. Priming can thus *direct* behavior towards pursuing a primed reward over competing rewards. In a runway paradigm, rats ran faster to the goal box after having received pretrial priming stimulation in the start box (Gallistel, 1969; Reid *et al.*,

1973; Gallistel *et al.*, 1974; Stellar & Gallistel, 1975), thus demonstrating the *potentiating* effect of priming on the vigor of reward-seeking behavior.

Dopamine transmission has been found to contribute differentially to the reinforcing and priming effects of electrical brain stimulation. For example, dopamine receptor antagonism with pimozide produces an extinction-like decline in operant responding for electrical brain stimulation (Gallistel *et al.*, 1982). This study indicates that dopamine transmission is necessary for the reinforcing effect of electrical brain stimulation. In contrast, high doses of pimozide failed to block the priming effect of electrical brain stimulation (Wasserman *et al.,* 1982), thus questioning whether the priming effect depends on dopamine transmission.

In the present study, we investigated whether food delivered non-contingently at the start of a trial produces a priming effect on subsequent food seeking and, if so, whether this effect is altered by blockade of dopamine transmission. The priming effect of food was measured using standard operant chambers and then challenged with dopamine D1 receptor (D1R) or D2 receptor (D2R) family antagonists. Experiment 1 employed a within-subject design whereby all rats received low, middle, and high doses of the D1R antagonist, SCH23390, and separately the D2R antagonist, eticlopride. In experiment 2, a between-subjects design was used: separate groups of rats received high doses of either SCH23390 or eticlopride. One limitation of Wasserman *et al.'s* (1982) experiment is that pimozide binds to 5-HT_7 receptors ($5HT_7R$) in addition to D2Rs and D3Rs, and it has very low affinity for D1Rs. In the present study, we employed more selective dopamine receptor antagonists to better discern the role of dopamine transmission in the priming effect. Thus, we examined whether the priming effect extends to a paradigm that uses standard operant conditioning chambers, whether food elicits a priming effect and, if so, whether this effect endures following treatment with highly specific dopamine receptor antagonists.

2. Method

2.1 Subjects

Forty-six male Long-Evans rats (in-house breeding colony, Concordia University, Montreal, QC) were used in experiments 1 and 2, respectively. Rats were housed in polyurethane shoebox cages, in a colony maintained at 23ºC, with a reverse 12-hour light/dark cycle (lights off from 0900 to 2100h). Initially, rats were pair-housed and had unrestricted access to standard lab chow and water. At the start of the experiment, rats were single-housed and food restricted to

90% of their free-feeding body weight. The rats weighed between 420 to 500 g throughout the experiment. All behavioral procedures were conducted during the dark phase of the diurnal cycle (between 1000 to 1400h). The protocols used were in accordance with guidelines established by the Canadian Council on Animal Care and approved by the Concordia University Animal Research Ethics Committee.

2.2 Apparatus

Tests were conducted in standard operant-conditioning chambers (30 cm length x 33 cm height x 27 cm width) each located within a fan-ventilated, sound-attenuating box. The chambers had aluminum walls and ceiling and a Plexiglas® front panel; the floors consisted of stainless-steel bars mounted 3 cm above the base. On the right wall, there was a house light (28 cm above floor), retractable lever (Coulbourn Instruments, Whitehall, PA), and a food port (1 cm length x 1.5 cm height). A nosepoke into the food port was detected by an infrared photocell beam. Connected to the outer right side of the chambers was an automated food dispenser that delivered chocolate pellets (45 g each, Bio-Serv, Pleasant Prairie, WI) into the food port. The apparatus was controlled by Graphic State 3.0 software (Coulbourn Instruments).

2.3 Behavioral Procedures

Habituation. Rats were habituated to the chambers for 20 minutes (min) per day for four consecutive days. Chocolate pellets were placed in the food port to associate that area with food reward. The last habituation day was followed by a rest day and then training.

Training. Rats were trained on a 1-second (s) fixed interval (FI1) schedule and progressed to FI2, FI4, FI6, FI8, and FI10 schedules consecutively. During the FI1 training sessions, a powdered form of the chocolate pellets was placed on the levers to motivate the rats to approach and press them. After a response was rewarded with a single chocolate pellet, the lever retracted, and the rat had to nose poke to trigger the re-extension of the lever. Throughout the training session, the house light remained on and was only briefly turned off during the delivery of the reward. For each operant schedule, rats were required to achieve the criterion of 50 lever-presses in a single training session on a given FI schedule before progressing to the next FI schedule. For example, if a rat produced 60 responses on its first training day on FI2, the next day the rat was trained on FI4. Generally, rats achieved criterion in one to two days. Each training session lasted 45 min. After rats learned to lever press on the FI10 schedule, they were introduced to one set of testing procedures. In the training trials, data was not collected because the sessions were for not

for testing purposes.

Testing.

Experiment 1. The parameters used in this experiment (e.g., the number of food primes, ITI, delay between priming and trial onset) were chosen based on preliminary tests that showed a priming effect of food was demonstrated with these conditions. A testing session started with a five-min inter-trial interval (ITI) during which the lever was retracted. On primed trials, three chocolate pellets were delivered at the end of the ITI. Once a nose poke was made, the rat was allowed 18 s to consume the pellets. The end of the consumption period activated the extension of the lever, which was armed on an FI10 schedule. A single response after the expiry of the FI triggered the delivery of one chocolate pellet and started a new ITI. Non-primed trials were similar with the exception that no chocolate pellets were delivered after the ITI. A single session consisted of three pairs of alternating primed and non-primed trials. These tests were run daily on sets of four test days. On test days one and three, the tests started with a primed trial. On test days two and four, the tests started with a non-primed trial.

Experiment 2. After experiment 1 and before experiment 2 was conducted, additional preliminary tests were carried out to optimize the reliability of the priming effect. It was found that the likelihood of a priming effect was increased by lengthening the time provided for pellet consumption to 30 s, allowing water consumption between trials, and starting tests with a primed trial. Thus, testing in experiment 2 was carried out identically to experiment 1 with the exception that rats were given 30 s to consume the pellets, water bottles were made available in the operant chambers, and all tests started with a primed trial. See Figure 1 for an outline of testing procedures.

2.4 Dopamine Receptor Antagonists

Experiment 1. Physiological saline (0.9 %), or the D1R antagonist SCH23390, or the D2R antagonist eticlopride (Sigma, St. Louis, MO), were administered intraperitoneally (i.p.) 30 min prior to the start of a test, on four consecutive days. Each set of tests with a dopamine receptor antagonist was followed by a rest day to wash out residual effects of the drug and then by four consecutive days on which saline was administered. The drug doses were increased across successive 5-day blocks so as to obtain a dose-response curve (0.01, 0.05, and 0.075 mg/kg dissolved in 0.9% saline).

These doses were selected based on preliminary experiments in our lab on the range of doses that reliably modifies operant responding for food. Doses higher than 0.075 mg/kg prevented the rats from completing the task. In addition, an attempt was made to test the rats in this paradigm using both D1R and D2R blockade. However, when both drugs were combined, even at the lowest doses, rats showed catatonic-like behavior, were unresponsive, and did not complete the task. Thus, a combined drug condition was not included in this study.

Experiment 2. All conditions were the same as in experiment 1, with the exception that only the high doses of SCH23390 and eticlopride were administered, and no rat received more than one type or dose of drug. Rats first completed a set of tests with saline. Rats that showed a priming effect with saline were administered a high dose (0.075 mg/kg) of SCH23390 or eticlopride.

2.5 Statistical Analyses

In experiment 1, data from one rat were removed because the operant chamber was later found to deliver pellets inconsistently. Therefore, the final *n* was nine. In experiment 2, data from 22 rats were excluded because they failed to show a priming effect with saline. Only rats that showed a priming effect with saline were tested with SCH23390 or eticlopride. Therefore, in experiment 2, six rats completed tests with SCH23390 and eight rats completed test with eticlopride.

Mean number of lever presses were calculated during the FI and the rate of responding was averaged over the set of four test days for each rat. Then mean total number of lever presses was normalized. The initial set of tests conducted with saline were used as baseline. A directional effect was hypothesized in that more vigorous responding was expected on the primed trials. Thus, planned, one-tailed paired-samples *t*-tests (alpha level = .05) were conducted on the normalized data. Effect sizes were measured with Cohen's *d*. Effect sizes have not been reported in previous research on the priming effect of pretrial reward delivery, and thus the standard interpretations for Cohen's *d* were adopted (Nolan & Heinzen, 2014).

Statistical analyses were conducted with JASP open-source statistics program (JASP Team, Version 0.8.5, Amsterdam, The Netherlands), and figures were created with GraphPad Prism (GraphPad, La Jolla, CA).

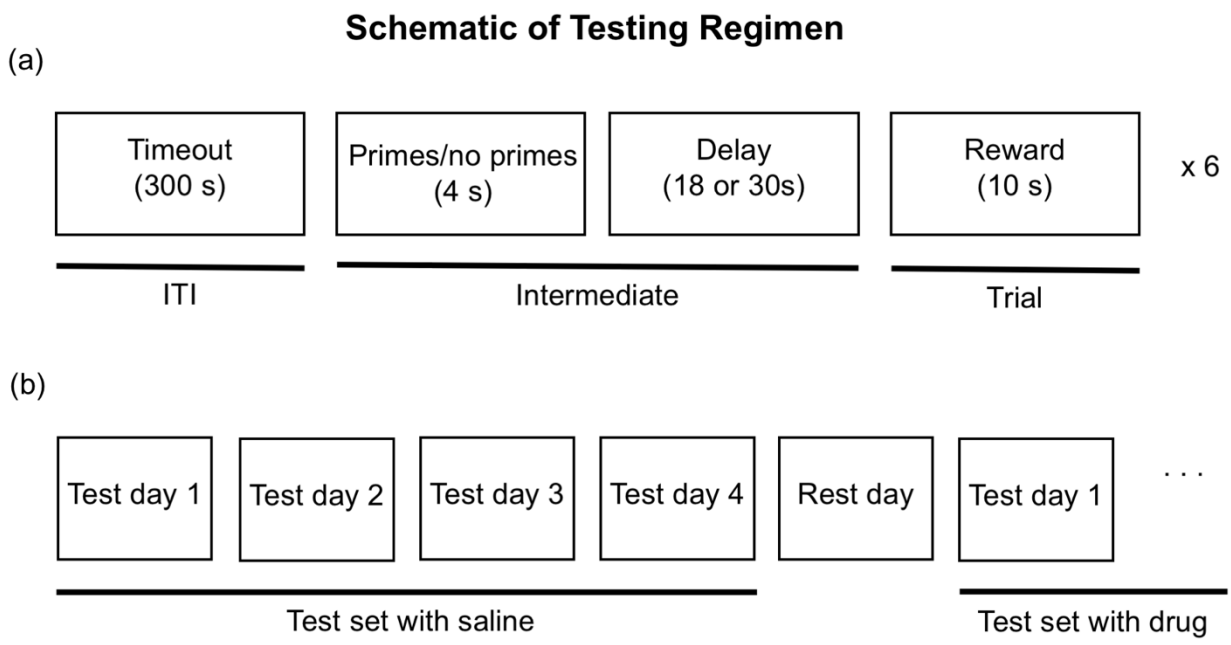

Figure 1. A schematic of a single trial and a 4-day set of tests followed by a rest day. (a) Preceding a trial was an intertrial interval (ITI) and an intermediate period. On primed trials, food primes were delivered during the priming phase of the intermediate period. Upon nose entry to the food magazine to consume the prime, the delay started. On non-primed trials, zero primes were delivered. Extension of the reward lever marked the start of the trial. A response made after the 10-s fixed interval resulted in delivery of a food reward, which was followed by a new ITI. A single test was composed of six trials; thus, this sequence of events was repeated six times. There were three primed and three non-primed trials, presented in alternating order. Values in parentheses represent the duration of the phase. (b) Data were collected in sets of four test days. For example, when rats were tested with saline, they were administered saline for four consecutive days. Completion of a 4-day set of tests was followed by a rest day to give the rats a break from testing and to serve as a washout period. After the rest day, a new 4-day set of tests started. If a drug was administered, the same drug condition and dose was used throughout the 4-day set of tests.

3. Results

3.1 The Priming Effect of Food

The present study demonstrates a priming effect of food (Figures 2, 3, & 5). In experiment 1, rats pressed more on primed than non-primed trials following saline treatment. According to the conventional interpretation of Cohen's *d*, this constitutes a medium to large effect ($t(8) = 2.024$, $p = .039$, Cohen's $d = .675$, Fig. 2a & 3a). Despite this overall priming effect, not all nine rats showed a priming effect. Six out of nine rats did so, and thus, 33% did not. Only 38% of rats showed a priming effect of food following saline in experiment 2. When present, the priming effect was large ($t(13) = 5.215$, $p < .001$; Cohen's $d = 1.394$; Fig. 5a). Thus, under the conditions tested here, a priming effect of food is observed but only in a select number of rats.

3.2 Dopamine Receptor Antagonists

Experiment 1. All nine rats were administered all three doses of and both types of dopamine receptor antagonists. At the highest dose of SCH23390, rats pressed more during primed than non-primed trials, and this was a medium to large effect (0.075 mg/kg, $t(8) = 2.217$, $p = .033$, Cohen's $d = .712$; Figures 2d & e). However, there were no statistically reliable differences between the normalized mean total lever presses on primed and non-primed trials at the lowest and middle doses of SCH23390 (0.01 mg/kg, $t(8) = .423$ $p = .342$, Cohen's $d = .141$; 0.05 mg/kg, $t(8) = 1.079$, $p = .156$, Cohen's $d = .360$; Figures 2a, b, & e).

Following treatment with eticlopride, responding was higher on primed than non-primed trials at the lowest and highest doses (0.01 mg/kg, $t(8) = 3.335$, $p = .005$, Cohen's $d = 1.112$; 0.075 mg/kg, $t(8) = 1.859$, $p = .049$, Cohen's $d = .620$; Figures 3b, d, e). These were large and medium effects, respectively. There was no significant priming effect following the middle dose (0.05 mg/kg, $t(8) = 1.794$, $p = .055$, Cohen's $d = .598$; Figures 3c & e).

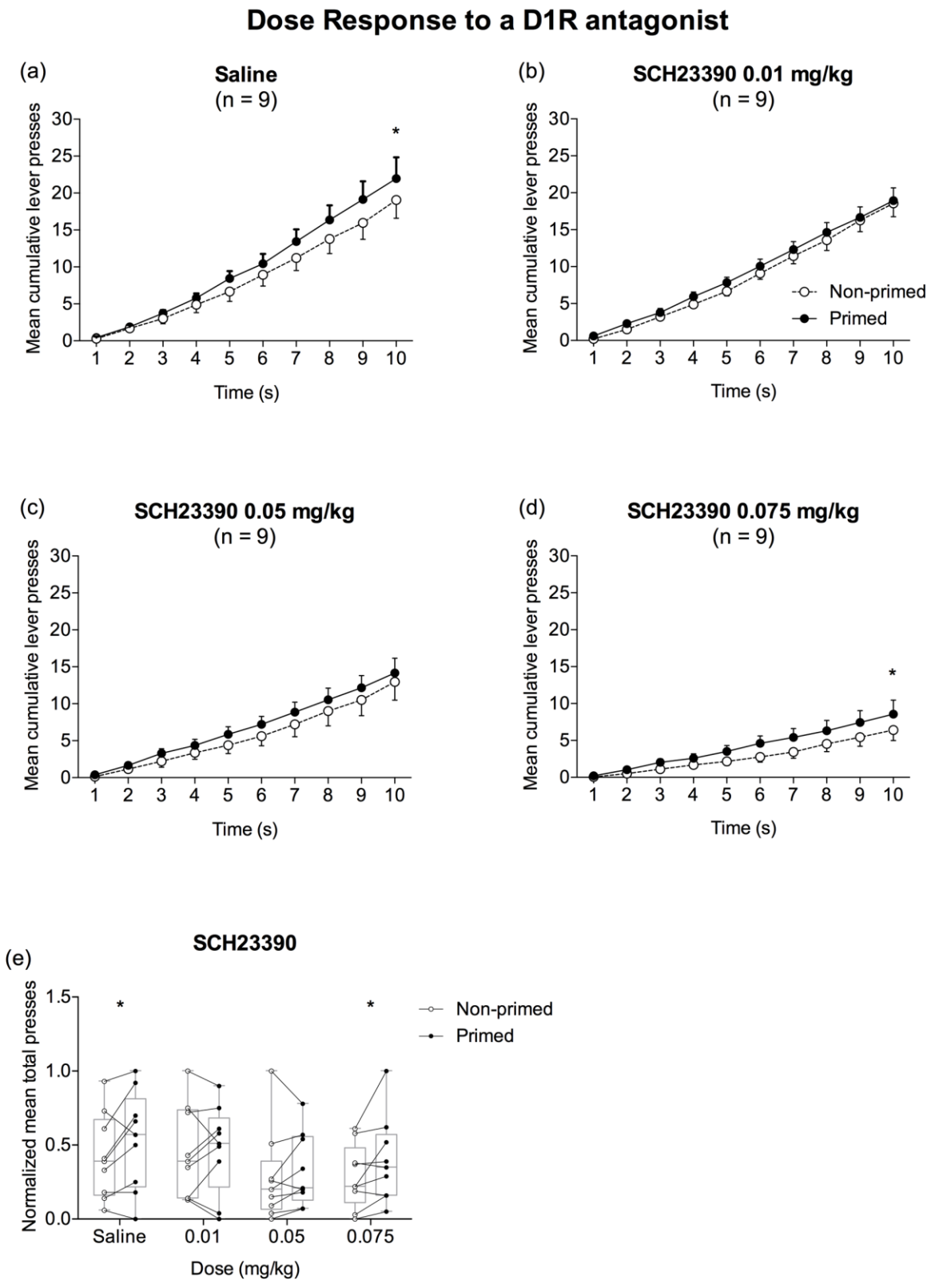

Figure 2. In experiment 1, a higher dose of the dopamine D1-like receptor antagonist SCH23390 does not eliminate the priming effect of food. Mean cumulative responses (± SEM) at the end of

the 10-s fixed interval with administration of (a) saline, (b) 0.01, (c) 0.05 or (d) 0.075 mg/kg of SCH23390. Box and whisker plots showing (e) the normalized mean total presses on non-primed and primed trials following saline and the three doses of SCH23390. Planned comparisons showed a significant difference between normalized mean total presses on primed versus nonprime trials following saline, and the 0.075 mg/kg dose of SCH23390.

* indicates statistically significant difference ($p < .05$)

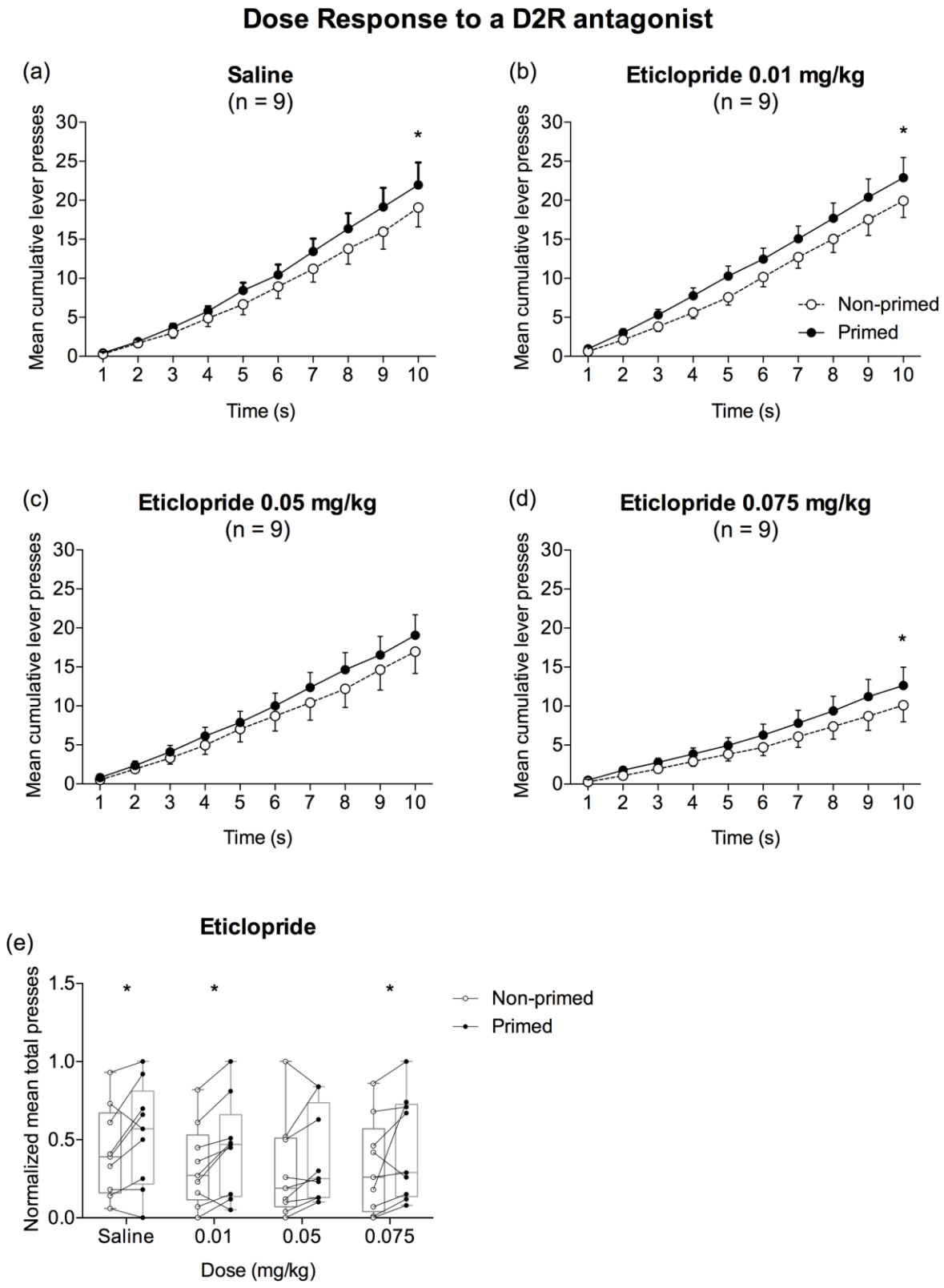

Figure 3. In experiment 1, dopamine D2-like receptor antagonism with eticlopride does not eliminate the priming effect of food. Mean cumulative responses (± SEM) at the end of the 10-s

fixed interval with administration of (a) saline, (b) 0.01, (c) 0.05 or (d) 0.075 mg/kg of eticlopride. Box and whisker plots showing (e) the normalized mean total presses on nonprime and primed trials following saline and the three doses of eticlopride. Planned comparisons showed a significant difference between normalized mean total presses on primed versus non-primed trials following saline and the 0.01 mg/kg and 0.075 mg/kg dose of eticlopride.
* indicates statistically significant difference ($p < .05$)

Dopamine Receptor Antagonism & Attrition Rate

In experiment 1, the proportion of trials completed across the set of tests was calculated for each drug condition and dose to assess the potential effects of dopamine receptor antagonism on motivation to complete a trial. The proportion of completed trials decreased as the drug dose increased when either SCH23390 or eticlopride was administered repeatedly (Fig. 4). When the dopamine receptor antagonists were administered just once in experiment 2, rats completed all the trials.

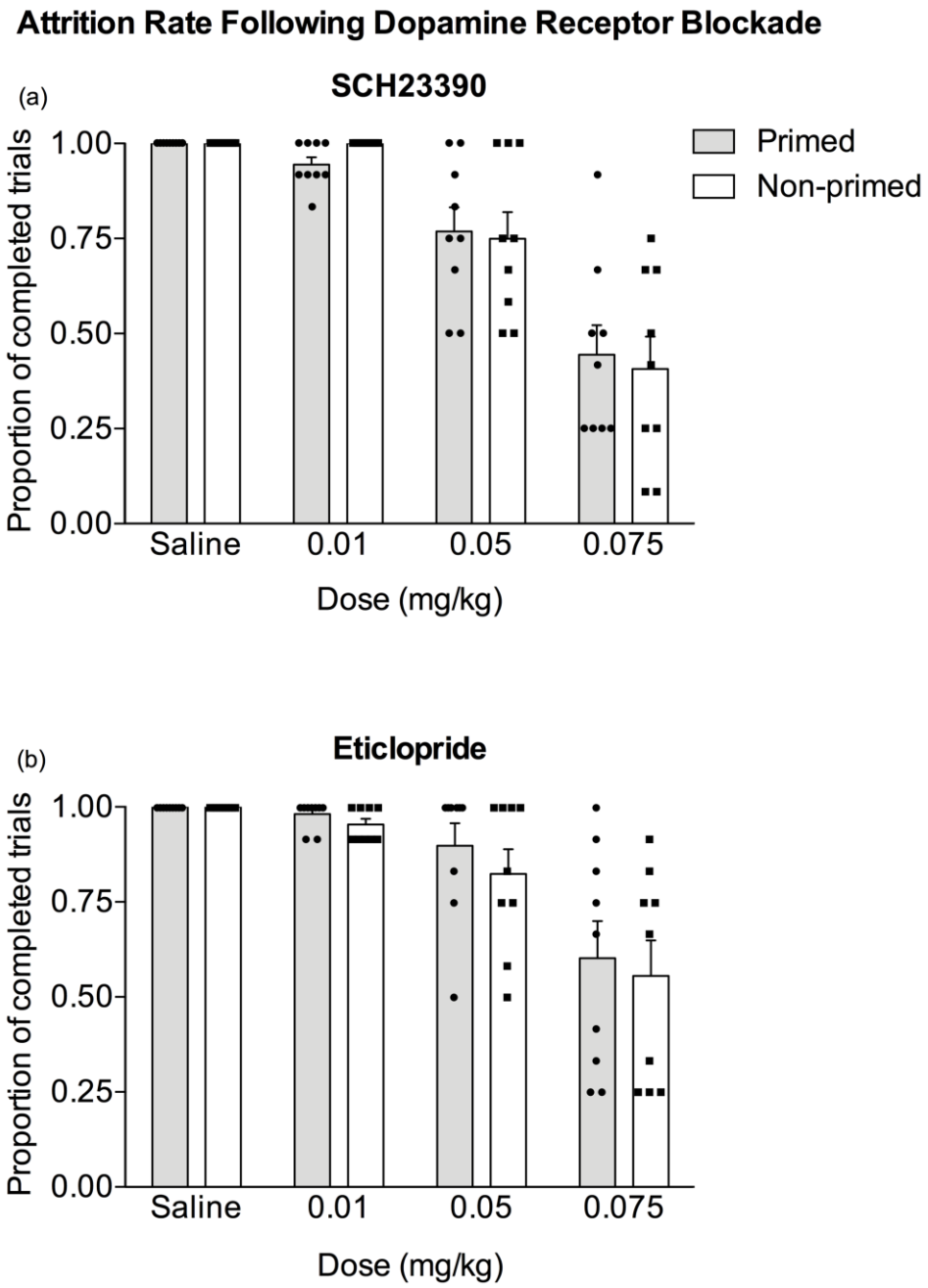

Figure 4. In experiment 1, attrition rate increases with higher doses of dopamine D1- and D2-receptor family antagonists. Proportion of completed trials (± SEM) for the saline condition and each drug dose of (a) SCH23390 and (b) eticlopride.

Experiment 2. In experiment 1, all rats received the three doses of both SCH23390 and eticlopride at different times, which may have produced confounding order effects. Thus, in experiment 2, separate groups of rats were tested with a high dose (0.075 mg/kg) of only either SCH23390 or eticlopride. Only those rats that showed a priming effect following saline were then administered a dopamine receptor antagonist. Following treatment with SCH23390, rats responded more on primed compared to non-primed trials ($t(5) = 2.042$, $p = .049$, Cohen's $d = .834$, Fig. 5b & d). The priming effect of food persisted with eticlopride ($t(7) = 2.868$, $p = .012$, Cohen's $d = 1.014$, Fig. 5c & d).

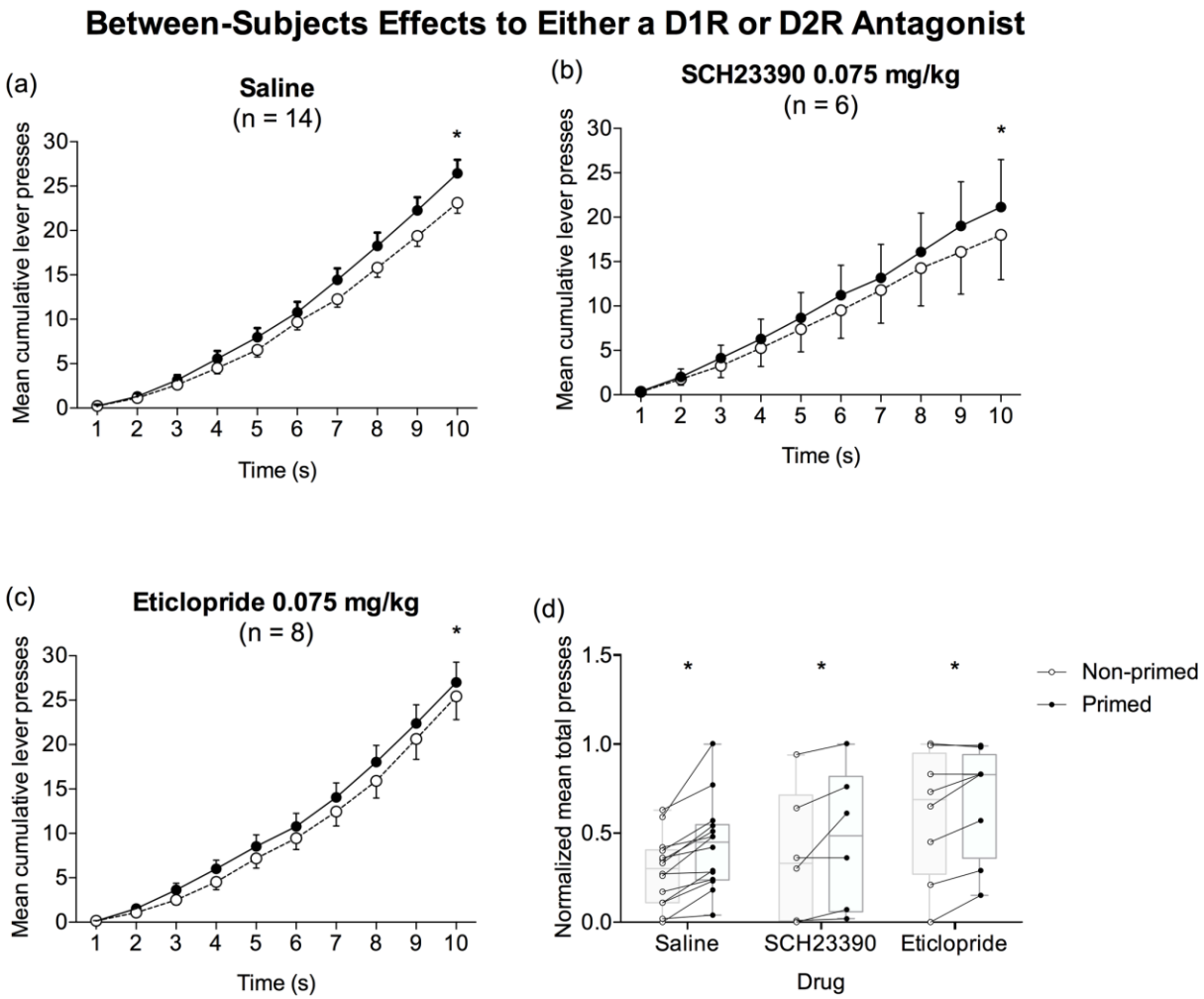

Figure 5. In experiment 2, the priming effect of food persists following dopamine D1- or D2-like receptor antagonism in a between-subject design. Mean cumulative responses (±SEM) at the end of the 10-s fixed interval with administration of (a) saline, or 0.075 mg/kg of (b) SCH23390 or (c) eticlopride. Box and whisker plots showing (d) the normalized mean total presses on non-primed and primed trials following saline, SCH23390 and eticlopride. Planned comparisons showed a significant difference between normalized mean total presses on primed versus non-primed trials following saline and both dopamine receptor antagonists.

* indicates statistically significant difference ($p < .05$)

4. Discussion

The priming effect investigated in the present study is the enhanced vigor of reward seeking in the wake of prior consumption of a reward. This effect is well-established in the case of electrical brain stimulation (Gallistel *et al.*, 1974; Edmonds & Gallistel, 1974; Stellar *et al.*, 1975). In contrast, there is inconsistent evidence as to whether an analogous effect is produced by natural rewards. This study reduces that ambiguity by demonstrating potentiated pursuit of food reward following pretrial delivery of food. Furthermore, like the priming effect of electrical brain stimulation, this priming effect of food is not eliminated by dopamine receptor antagonism. This finding is not readily explained by hypotheses such as the incentive salience hypothesis, which posits that the incentive motivational effect of rewards, called "wanting", is mediated by dopamine transmission.

4.1 The Priming Effect of Food Reward

Previous work on the priming effect of food was carried out largely in mazes or runways. van der Kooy and Hogan 1978 primed hamsters by allowing them to consume food in each corner of a rectangular maze prior to traversing a maze segment leading to the next corner; the type and level of deprivation was varied, as was the ITI. The fastest running speeds were recorded at the shortest ITI (10 s), a result that qualifies as a priming effect. In an initial study, Terry (1980) administered food primes 0.5 or five min before rats were given access to a runway leading to food. A priming effect was seen at the 0.5-min delay on the first day but then largely disappeared with further training.

A few studies have used standard operant method for investigating the priming effect of food. Deluty (1976) trained rats to press a lever for food on a one-min random interval (RI) schedule, and delivered food primes on a fixed or random time (FT or RT) schedule. Responding decreased as the reward rate on the FT or RT schedule increased, a finding that could be interpreted as an anti-priming effect: an inhibition of subsequent reward seeking following reward delivery. The observed effect could instead be due to satiation driven by the high reward rate. Responding increased immediately after the delivery of primes and then decreased as a function of time since the last prime, a time course consistent with the transient nature of the priming effect. Eiserer (1978) trained rats to lever press for a single food pellet and determined whether food priming facilitated reinforced or non-reinforced responding. Even when lever

pressing was reinforced with food, there were periods of time when the rats ceased to respond. Delivery of a food prime increased the probability that responding would resume.

In the present study, a standard operant design was used to measure the priming effect of food. Previous experiments and preliminary tests helped identify parameter values suitable for measuring a priming effect of food: six trials per test, 5-min ITIs, three food primes, and an 18-s delay between delivery of food primes and trial onset in experiment 1. Restricting the number of trials per test to six and setting the ITI to 5 min helps slow the onset of satiation, which are in accordance with the methods of van der Kooy and Hogan (1978) and Terry (1983). In previous food priming studies, rats were primed with one to five pellets (Deluty, 1976; Eiserer, 1978; Terry, 1980, 1983). The median value of three pellets was used in the present study to avoid quickly satiating the rats. A delay of 18 s was employed to permit the rats substantial time to consume the food primes and to allow time for the primes to take effect. Deluty (1976) found that a single food prime maximally increases responding 10 s after the delivery of the primes. He reasoned that the rats need 10 to 15 s to approach the food port and consume the three food primes and then an additional 10 s for the primes to take effect. The 18-s delay employed in experiment 1 met those criteria. In experiment 2, the delay was increased to 30 s because preliminary data from our lab that suggested allowing the animals more time to consume the food primes was more likely to produce a priming effect of food.

Baseline tests with saline injections demonstrated that the chosen parameters yield a priming effect of food. As predicted, the rats in experiment 1 responded more during the 10-s FI on primed compared to non-primed trials (Figures 2 & 3). Rats in experiment 2 also showed a priming effect of food even with the longer delay between priming and trial onset (Figure 5). These results validate the use of this operant design for investigating the role of dopamine transmission in the priming effect of food.

4.2 Variability of the Priming Effect of Rewards

It is noteworthy to consider that the priming effect of food was not seen in every rat. This is in contrast with the 100% incidence of a priming effect of electrical brain stimulation observed in a runway paradigm (Reid *et al*., 1973). Although the magnitude of the priming effect varied among rats, Gallistel reported a robust priming effect of medial forebrain bundle stimulation. In comparison, six of nine rats showed a priming effect following saline administration in experiment 1, and 14 out of 36 rats showed a priming effect in experiment 2. Because of this, in

experiment 2, only the rats that showed a priming effect with saline were later tested with SCH23390 or eticlopride.

Some of the previous work on the priming effect of food and drugs of abuse have also demonstrated variability in the incidence of the priming effect. In one experiment, Terry (1980) observed a priming effect of food following a 0.5-min delay between consumption of the food primes and start of a trial compared to a 5-min delay. However, this priming effect was specifically observed only on the first day of training. In another study, the detection of a priming effect depended on whether comparisons were made within or between subjects (Terry, 1983). Rats were either primed with food immediately before the start of a trial or not primed. Between-subject comparisons showed no priming effect. On the other hand, within-subject comparisons revealed that rats were faster to run when they were primed.

Observation of a priming effect of alcohol has also been variable. Chutuape *et al.* (1994) examined the priming effect of alcohol in social drinkers. Participants were given the opportunity to perform tasks that earned them money or alcohol. The probability of winning money varied from low to high, and the probability of winning an alcoholic beverage was constantly moderate. Priming social drinkers with alcohol led them to work more for alcoholic beverages when the probability of earning money was low. Alcohol-primed social drinkers also reported increased desire for alcohol and liking of alcohol. In another experiment, Kirk and de Wit (2000) used the same methods as above and replicated the finding that priming social drinkers with alcohol increases reports of liking alcohol and desire to consume more alcohol. However, they did not find that priming with alcohol increased probability of choosing alcohol over money in the choice task. The observation of a priming effect of alcohol in social drinkers was found to be modulated by individual differences.

In summary, the incidence of a priming effect of rewards appears to be more variable when food and drugs of abuse, rather than electrical brain stimulation, serve as the reward. Individual preferences for certain rewards may contribute to the occurrence of a priming effect. In support of this idea, Kirk and de Wit (2000) found a strong priming effect of alcohol in social drinkers who experienced positive changes in mood from alcohol consumption. A preferred reward can elicit positive effects on mood and lead to overconsumption of the reward, whereas a less preferred reward is less likely to elicit such effects (Kampov-Polevoy *et al.*, 2006).

4.3 Dopamine and Performance

There is a large literature on the role of dopamine neurons in the reinforcing and motivating effects of rewards (Franklin, 1978; Wise, 1978, 1996; Wise & Rompre, 1989; Ikemoto & Panksepp, 1999) but, to our knowledge, there has only been a single paper published on the role of dopamine transmission in the priming effect of rewards (Wasserman *et al.*, 1982). Much of the early literature used a one-dimensional approach that addressed the degree to which the changes in operant performance observed following perturbation of dopamine transmission reflect alterations in reward strength or changes in response capacity. A curve-shift paradigm that measures performance over a range of reward strengths was developed to distinguish these two effects in studies of intracranial self-stimulation. Proponents of the curve-shift method claim that lateral displacement of curves that relate response vigor to the strength of the electrical stimulation reflect changes in its reward strength, whereas changes in the upper asymptote of the curve (e.g., the maximum response rate) reflect alterations in response capacity (Edmonds & Gallistel, 1974; Miliaressis *et al.*, 1986).

Extension of the testing paradigm to a third dimension, reward cost, shows that the lateral displacements observed in a curve-shift paradigm are ambiguous and may be due to various combinations of changes in reward-system sensitivity, reward-system gain, subjective effort cost, or the value of alternate activities such as resting, grooming and exploring (Arvanitogiannis & Shizgal, 2008; Hernandez *et al.*, 2010; Breton *et al.*, 2013). The behavioral effects produced by perturbation of dopamine transmission in the present experiment point to some combination of the latter three effects (Hernandez *et al.*, 2010, 2012; Trujillo-Pisanty *et al.*, 2014). Any of these effects, or a combination thereof, could account for the failure of the rats to complete all trials at the higher drug doses in experiment 1 (Figure 4). Continued responding would no longer be consistently worthwhile in the face of sufficient decreases in reward-system gain, increases in the perceived effort entailed in pressing the lever, and/or increases in the value of competing activities.

A common finding in studies of operant performance for natural or electrical rewards is that maximum response rates are decreased by treatment with dopamine receptor blockers. For example, eticlopride impairs motor activity at higher doses (Garrett & Holtzman, 1994; Collins *et al.*, 2010), and SCH23390 does so even at low doses (Daniela *et al.*, 2004; Pezze *et al.*, 2015).

In experiment 1, antagonist-induced motor impairment could account for the decrease in the number of responses (Figures 2 & 3) and the reduced number of completed trials (Figure 4).

4.4 Dopamine and the Priming Effect

What is striking about the present results is that the priming effect was largely able to ride on top of the drug-induced decreases in responding. Although responding was attenuated, especially in experiment 1 following higher doses of the dopamine receptor antagonists, pretrial delivery of food pellets continued to augment response vigor. It would have been interesting to show synergistic effects of combining the dopamine D1- and D2-like receptor antagonists. However, when we did this, rats became unresponsive and did not complete the task. Nevertheless, the persistence of the priming effect in the face of drug challenge is seen following administration of dopamine D1- or D2-like receptor antagonists. Taken together, the results are consistent with what Wasserman *et al.* (1982) observed using a different testing paradigm (a runway), a different reward (medial forebrain bundle stimulation), and a different drug (pimozide), one with a broader and partially non-overlapping spectrum of action (Beaulieu & Gainetdinov, 2011).

Gallistel and his team acquired a substantial body of evidence that distinguishes the reinforcing effect and priming effect of electrical brain stimulation (Gallistel, 1969; Gallistel *et al.,* 1974; Edmonds & Gallistel, 1974). The work of both Deutch's and Gallistel's teams showed that the priming effect shares the two key attributes of motivation: it both directs and potentiates subsequent reward-seeking behavior (Deutch *et al.*, 1964; Wasserman *et al.*, 1982). Echoing and extending the earlier work, the findings of the present study are consistent with the notion that the reinforcing effect and priming effect of rewards are distinct and are mediated by different neural mechanisms. The present study shows that like the priming effect of medial forebrain bundle stimulation, the priming effect of food is insensitive to attenuation of dopamine transmission. Pimozide, a drug that has high affinity for D2Rs, D3Rs, and serotonin 5-HT_7Rs (Richelson & Souder, 2000; Kroeze *et al.*, 2003; Burstein *et al.*, 2005), was used by Wasserman *et al.* (1982) in their study of the priming effect produced by medial forebrain bundle stimulation. The more specific drugs employed in the present study made it possible to demonstrate that the priming effect of food remains largely or wholly intact following selective blockade of D1Rs or D2Rs at doses sufficient to produce reductions in performance and failures to seek out food rewards.

The insensitivity of the priming effect to antagonism of D1Rs and D2Rs is surprising in view of the incentive salience hypothesis, which posits that incentive motivational effects of reward ("wanting") are mediated by dopamine transmission (Robinson & Berridge, 1993; Berridge & Robinson, 1998). A broader view of the neural substrates for motivation is required to accommodate the empirical findings on which the incentive salience hypothesis is based along with the present results and those obtained by Wasserman *et al.* (1982). An extended view of the neural bases of motivation that could encompass all of these findings would include multiple converging pathways that direct and invigorate reward-seeking behavior. The set of pathways in question appears to include both dopaminergic and non-dopaminergic elements.

5. Conclusion

Understanding the neurobiological basis of the priming effect of rewards holds important implications for disorders associated with impaired motivation such as binge eating. For instance, the taste of a preferred food can trigger a person with a binge-eating disorder to overconsume. Elucidating the neurobiological mechanisms underlying the priming effect of rewards could help break the cycle of overconsumption. Although there has been a large body of research that focuses on the role of the dopamine system in reinforcement and motivation, there is still a lack of effective treatments for disorders associated with impairments in motivation. Perhaps this focus on the dopamine system has obscured the complementary roles of other neurotransmitter systems. This emphasizes the need to reconsider the role of non-dopamine systems in reward-related processes such as motivation.

Chapter 5: General Discussion

Dopamine transmission has been a focal point of research on reward and motivation. In this thesis, reward describes the propensity of goal objects, such as electrical brain stimulation and food, to promote approach. Motivation is defined in terms of the direction and invigoration of goal-seeking behavior. Although the importance of the midbrain dopamine system in reward and motivation has been well documented, there is still no universally agreed upon neurobiological theory for dopamine's role in reward seeking. The contributions of other neurotransmitter systems in reward and motivation may be important to consider. Our research on the priming effect of electrical brain stimulation and food indicate that priming may not depend on dopamine signaling.

This thesis investigated the priming effect of electrical brain stimulation and food and the role of dopamine transmission. The priming effect has primarily been studied using electrical brain stimulation as a reward. Expanding on this work, Chapter 2 investigated whether the priming effect of electrical brain stimulation is affected by reward strength, cost, or both. We showed that the priming effect of electrical brain stimulation is more likely to be observed when the payoff is high (i.e, when the reward is intense and inexpensive). In addition to this, we investigated whether the priming effect is specific to electrical brain stimulation or generalizable to other rewards. Chapter 4 demonstrates that a natural reward such as food also elicits a priming effect. Finally, Chapters 3 and 4 examined whether dopamine transmission is necessary for the priming effect of electrical brain stimulation or food, respectively. We demonstrated that dopamine D2 family receptor (D2R) antagonism does not eliminate the priming effect of electrical brain stimulation and food. Additionally, the priming effect of food persists following D1R blockade. Thus, we provide evidence that certain aspects of motivation, such as the priming effect of rewards, may not primarily depend on dopamine transmission.

1. Is the Incidence of a Priming Effect of Rewards Consistent?

The priming effect of electrical brain stimulation was previously reported in 100% of the rats tested (Reid *et al.*, 1973). In contrast, we found high variability in the incidence of a priming effect of electrical brain stimulation and food. In Chapter 2, a priming effect of electrical brain stimulation was seen in 33% of the rats in experiment 1 and 25% to 75% of the rats in experiment 2. In an attempt to derive a more consistent priming effect, the method used to measure a priming effect of electrical brain stimulation was modified in Chapter 3 to become

more analogous to the runway paradigm used by Gallistel and colleagues (Reid *et al.*, 1973; Edmonds & Gallistel, 1974; Gallistel *et al.*, 1974; Stellar & Gallistel, 1975; Wasserman *et al.*, 1982). Chapter 3 results showed that following high priming 88% of the rats tested were quicker to initiate a lever press and 100% of the rats tested worked faster to earn a reward. Comparable to Reid *et al.'s* (1973) results, the magnitude of the priming effect varied among rats. The method used in Chapter 3 produced the most consistent incidence of a priming effect of electrical brain stimulation in this thesis.

Another possible reason for the variability in the incidence of the priming effect of electrical brain stimulation could be that the runway paradigm is more sensitive to the motivational changes elicited by priming than the methods employed here. In experiment 1 of Chapter 3, the range of speed to initiate a response was as half a second to two seconds. The range of speed to earn a reward was three seconds to seven seconds. In the runway, the range of speed to earn a reward was approximately two to 20 seconds (Reid *et al*., 1973). Thus, the range for measurement is highly constricted in our paradigm. Additionally, reward procurement in the runway paradigm may require greater effort compared to our method, which may affect the sensitivity of that paradigm at detecting changes in motivation. Future experiments should examine whether a runway paradigm or other operant designs would be best suited for measuring the priming effect of electrical brain stimulation.

The incidence of a priming effect of food was also variable. In Chapter 4, 39% to 67% of the rats showed a priming effect of food. One possible reason for this variability is that the method we used to observe a priming effect of food requires further optimization. In the experiments conducted in Chapter 4, the lever was armed on a fixed interval (FI) schedule. The rate of reward delivery is little affected by changes in rate of responding on an FI schedule. In contrast, in the runway paradigm the rat has greater control of how soon reward can be harvested once it becomes available. That is, the faster the rat runs down the alley then the sooner it can earn the reward. Future experiments should examine whether the use of a ratio schedule, similar to the one used in Chapter 3, would result in a more consistent priming effect of food.

Another potential reason for the variability in the incidence of the priming effect of food may be due to the strength of the food reward. As demonstrated in Chapter 2, rats do not show a priming effect when the earned reward is weak. This issue was addressed in the electrical brain

stimulation experiments by customizing the stimulation parameters for each rat. Customizing the strength of food for each rat may improve the incidence of a priming effect of food.

It is also possible that the priming effect of food was not seen in all rats in this thesis simply because some rats are more sensitive to food priming than others. It is not uncommon that certain phenomena are observed only in a specific population. For example, not all people that use or consume drugs develop a drug use disorder. In 2017, it was reported by the National Survey on Drug Use and Health that 86.3% of persons aged 18 and older have consumed alcohol within their lifetime. Only 5.7% of that population reported an alcohol use disorder. This shows humans have individual differences in their responses to drugs of abuse.

Similar to humans, rats have demonstrated individual differences in their proclivity to seek drugs of abuse (for review see Piazza & Le Moal, 1996). One method used to study drug use and maintenance in rats is by training them to self-administer drugs of abuse, such as amphetamine. A drug is self-administered by performing an operant response, such as a nose-poke. Piazza *et al.* (1989) showed that only a subset of rats acquire amphetamine self-administration. Rats that show a strong locomotor response to a mild stressor more readily acquire amphetamine self-administration. Piazza *et al.* (1991) provided evidence that corticosterone contributes to the individual vulnerabilities to amphetamine self-administration. Locomotor response to a mild stressor positively correlates with stress-induced corticosterone release. Additionally, corticosterone administration facilitates amphetamine self-administration in rats that showed a low locomotor response to mild stressor. These studies show that behavioral and biochemical reactivity to a mild stressor predict individual vulnerabilities to drug use and maintenance in rats.

There are individual differences in incentive salience attribution, which is when cues paired with rewards lead to those cues acquiring incentive value or desirability, in rats. The incentive-sensitization theory proposes that compulsive reward seeking, such as drug addiction, results from excessive incentive salience attribution to reward-related cues (Robinson & Berridge, 1993). One method used to investigate incentive salience attribution is an autoshaping paradigm (Brown & Jenkins, 1968; Williams & Williams, 1969; Uslaner *et al.*, 2006). This involves classically conditioning rats to associate a cue with the availability of a reward. Upon presentation of a reward-predictive cue, one group of rats respond by approaching and

interacting with the cue (i.e., sign-tracking) and a separate set of rats ignore the cue and directly approach the location of the reward (i.e., goal-tracking) (Meyer *et al.*, 2012).

Sign-tracking is proposed to reflect excessive incentive salience attribution to reward-related stimuli. Rats that demonstrate sign-tracking show greater sensitization to cocaine (Flagel *et al.*, 2008) and acquire self-administration of cocaine at a low dose compared to goal-trackers (Beckmann *et al.*, 2011). Sign-trackers also show higher corticosterone levels and elevated dopamine concentration in the nucleus accumbens (NAc) (Tomie *et al.*, 2000). These results indicate individual differences in incentive salience attribution are correlated with biochemical activity and vulnerability to reward seeking.

Future studies should investigate which factors enhance individual susceptibility to the priming effect of food. To our knowledge, reward preference has been the only factor that has been correlated with individual differences in response to priming. Kirk and de Wit (2000) showed a strong priming effect of alcohol in social drinkers that experienced positive changes in mood from an alcohol prime. A preferred reward can elicit positive effects on mood and lead to overconsumption of that reward (Kampov-Polevoy *et al.*, 2006). Other factors could affect sensitivity to priming such as behavioral response to mild stressors, corticosterone levels, and disposition to attribution incentive salience to cues.

2. If Dopamine Isn't the Primary Mediator of the Priming Effect of Rewards, then What Other Neurochemicals Might It Depend On?

There is a rich literature that has documented the importance of dopamine transmission in reward and motivation (for reviews see Berridge & Robinson, 1998; Wise, 2004, 2006, 2008; Salamone *et al.*, 2009; Salamone & Correa, 2012; Walton & Bouret, 2019). For example, rewards such as food, drugs, and electrical brain stimulation are associated with increases in midbrain dopamine activity (Fibiger, 1978; Wise, 1978; Wise & Rompre, 1989; Fiorino *et al.*, 1993; You *et al.*, 2001; Phillips *et al.*, 2003; Roitman *et al.*, 2004; Rodeberg *et al.*, 2016). Additionally, the incentive salience hypothesis postulates *wanting*, but not *liking*, of reward is specifically mediated by dopamine transmission (Robinson & Berridge, 1993; Berridge & Robinson, 1998). Despite this, the present thesis provides evidence consistent with the idea that the priming effect of electrical brain stimulation and food may not depend on D2R signaling. We also provide evidence that suggests the priming effect of food does not depend on D1R signaling, but we cannot rule out the contribution of D1Rs in the priming effect of electrical brain

stimulation. This elicits an important question: If the priming effect of rewards does not depend on both D1Rs and D2Rs, then what neurochemical(s) might it depend on?

The medial forebrain bundle (MFB) has been highly implicated in reward. It consists of ascending and descending fibers that course through the olfactory regions to the brain stem (Nieuwenhuys *et al.*, 1982; Geeraedts *et al.*, 1990a, 1990b). Stimulating regions of the MFB has been shown to promote eICSS (Olds & Milner, 1954; Olds, 1956; Olds & Olds, 1963; Koob *et al.*, 1978; Phillips, 1984). The MFB contains a heterogenous population of neurons (McMullen & Almli, 1981; Nieuwenhuys *et al.*, 1982; Geeraedts *et al.*, 1990b, 1990a), and it remains unknown which subset of those neurons are responsible for eICSS.

The lateral hypothalamic (LH) level of the MFB has been one of the most commonly studied regions for eICSS (Wise, 1980, 1996; Gallistel *et al.*, 1981; Gratton & Wise, 1983; Yeomans *et al.*, 1985; You *et al.*, 2001). Parametric experiments have indicated that the LH-MFB fibers that are directly stimulated in eICSS are not dopamine neurons. The conduction velocity of the directly-stimulated fibers in the LH-MFB ranges from 1 to 8 m/s (Shizgal *et al.*, 1980; Bielajew & Shizgal, 1982; Murray & Shizgal, 1994, 1996a, 1996b) and the absolute refractory period ranges from 0.4 to 1.5 ms (Yeomans, 1979; Shizgal *et al.*, 1980; Bielajew *et al.*, 1982). In contrast, dopamine neurons have slower conduction velocities (0.28 to 1.00 m/s) and longer absolute refractory periods (1.2 to 2.5 ms) (Wang, 1981; Yeomans *et al.*, 1988; Anderson *et al.*, 1996). These findings indicate that the directly-stimulated LH-MFB fibers are myelinated and highly excitable while dopamine neurons are unmyelinated and require high thresholds for excitation.

The directly-stimulated LH-MFB fibers are predicted to transsynaptically activate dopamine neurons located in the ventral tegmental area (VTA) to support eICSS (Shizgal *et al.*, 1980; Wise, 1980; Gallistel *et al.*, 1981; Shizgal, 1989; Yeomans, 1989). VTA-dopamine neurons project to a variety of cortical and subcortical structures (Swanson, 1982; Lammel *et al.*, 2014; Beier *et al.*, 2015; Poulin *et al.*, 2018). In particular, their projection to the NAc is implicated in motivated behavior (Ikemoto & Panksepp, 1999; Phillips *et al.*, 2003; Ikemoto, 2007; Mohebi *et al.*, 2019). You *et al.* (2001) showed that stimulating LH-MFB neurons increases dopamine concentrations in the NAc. This finding supports the idea that midbrain dopamine, via transsynaptic activation, mediates eICSS. However, this cannot explain our finding that the priming effect does not depend on dopamine signaling.

Perhaps instead the priming effect is driven by non-dopamine neurons in the MFB. For example, neurons in the LH release a heterogenous population of neuropeptides (Godfrey & Borgland, 2019; Mickelsen *et al.*, 2019). One such neuropeptide called orexin (or hypocretin) is involved in motivated behaviors such as priming-induced reinstatement and self-administration of drugs and food (Harris *et al.*, 2005; Borgland *et al.*, 2009; Aston-Jones *et al.*, 2010). But orexin is most well-known for its role in arousal (de Lecea *et al.*, 1998; Sakurai *et al.*, 1998). Ren *et al.* (2018) showed that LH-orexin neurons that send inputs to paraventricular nucleus (PVT) glutamate neurons that project to the NAc are important for controlling arousal. Stimulation of arousal (or behavioral activation) is considered an aspect of motivation that energizes goal-directed behavior (as mentioned in Salamone *et al.*, 2007; Salamone & Correa, 2012). The energizing effect of priming may be mediated by LH-orexin neurons that send inputs to NAc-projecting PVT-glutamate neurons.

Alternatively, there is evidence that LH stimulation excites neurons in the amygdala (Rolls, 1972) and induces Fos protein expression there (Arvanitogiannis *et al.*, 1996). Kadar *et al.* (2011) showed that eICSS in the LH is associated with c-Fos protein expression in the basolateral amygdala (BLA). Glutamate projections from BLA to the NAc are important for cue-induced reward seeking (Di Ciano & Everitt, 2004; Ambroggi *et al.*, 2008). Stuber *et al.* (2011) showed that mice optically self-stimulate those glutamate fibers, and that behavior does not depend on D2Rs. This is consistent with our finding that the priming effect of electrical brain stimulation and food does not depend on D2R signaling. Electrical stimulation of the LH may transsynaptically activate BLA neurons, such as glutamate, that project to the NAc to drive the priming effect.

The VTA also contains a heterogenous population of neurons that express dopamine, GABA, glutamate (Yamaguchi *et al.*, 2007, 2011; Nair-Roberts *et al.*, 2008; Dobi *et al.*, 2010; Morales & Root, 2014). Some VTA neurons can co-release neurotransmitters (combinatorial neurons) such as dopamine and glutamate or dopamine and GABA (Stuber *et al.*, 2010; Mestikawy *et al.*, 2011; Roeper, 2013; Morales & Root, 2014; Yoo, Zell, Gutierrez-Reed, *et al.*, 2016; Morales & Margolis, 2017; Wang *et al.*, 2017). Another possibility is that priming is mediated by glutamate neurons in the VTA. There are populations of VTA-glutamate neurons that respond to aversive or rewarding stimuli, or both (Root *et al.*, 2018). Two separate groups of researchers showed that optical activation of VTA-glutamate neurons that project to the NAc can

promote avoidance or approach. Qi *et al.* (2016) demonstrated that mice avoid a chamber paired with optical stimulation of VTA-glutamate neurons and that they will work to terminate VTA-glutamate optical stimulation. In contrast, Yoo *et al.* (2016) showed that mice optically self-stimulate VTA-glutamate neurons. These discrepant findings could be due to differences in their photostimulation settings. Qi *et al.* (2016) used continuous or prolonged photostimulation of VTA-glutamate neurons, which may be aversive. Yoo *et al.* (2016) showed that mice prefer to self-stimulate for brief VTA-glutamate photostimulation. In the present thesis, brief pulse-trains were used. This may have led to a brief, transsynaptic stimulation of VTA-glutamate neurons to produce a priming effect.

The MFB fibers that project to the hindbrain are another possible substrate for the priming effect. Pritzel *et al.* (1983) conducted a study whereby an electrode was implanted in the LH level of the MFB, the forebrain ipsilateral to the stimulation site was removed, and the striatum contralateral to the stimulation site was isolated from the brain stem. These manipulations largely, but probably not completely, removed or disconnected the basal forebrain terminal fields of the midbrain dopamine neurons. Despite this, rats still behaved for LH-MFB stimulation. This finding suggests that a subset of non-dopamine neurons important for reward are located in the hindbrain, and those neurons may be involved in the priming effect.

Results from this thesis indicate that the priming effect of rewards may be mediated by non-dopamine systems. Here, we speculated that the priming effect may be mediated by non-dopamine neurons in the MFB such as 1) LH-orexin neurons that project to PVT-glutamate neurons that send inputs to the NAc, 2) LH fibers that synapse with BLA-glutamate neurons that project to the NAc, 3) VTA-glutamate neurons that send inputs to the NAc, and/or 4) MFB fibers in the brain stem (Figure 1). This is only a selection of circuits potentially involved in the priming effect; thus, many others not mentioned here could also be important for priming.

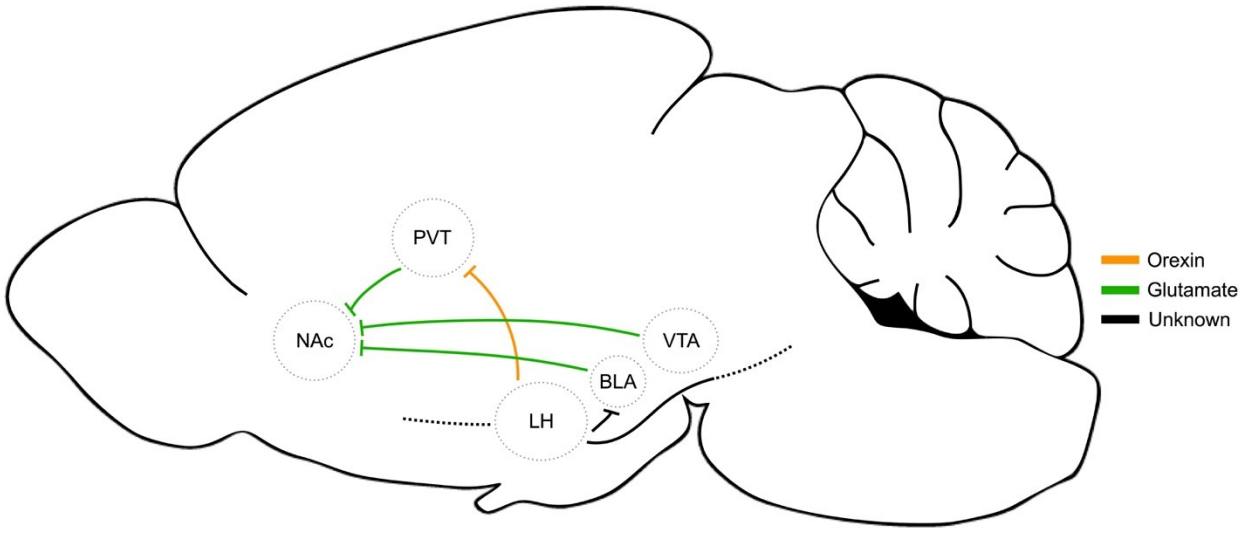

Figure 1. Non-dopamine circuits proposed to be involved in the priming effect of rewards. Priming may activate non-dopamine neurons in the MFB such as 1) LH-orexin neurons that project to PVT-glutamate neurons that send inputs to the NAc, 2) LH fibers that synapse with BLA-glutamate neurons that project to the NAc, 3) VTA-glutamate neurons that send inputs to the NAc, and/or 4) MFB fibers in the brain stem. The legend shows that orange fibers represent orexin neurons, green fibers represent glutamate neurons, and black fibers represent population of neurons whose neurochemical identity is unknown. The dotted portion of the fibers projecting from the LH indicate that those fibers may project to variety of hindbrain structures and may arise rostral to the LH.

A caveat to these speculations on the neurobiological bases of priming is that the studies mentioned previously do not directly investigate the priming effect. The reward-related behavioral measures quantified preference (e.g., real-time place preference) or operant behavior (e.g., optical self-stimulation). The neural circuits we speculated to mediate the priming effect may be involved in those reward-related behaviors but not the priming effect. To determine which neural circuits are involved in priming, future studies should investigate whether activation of those circuits is sufficient and necessary to elicit a priming effect.

It is also important to keep in mind that behaviors can have multiple causes, which is the problem of convergent causation. For example, conditioned-place preference and ICSS have been shown to involve both dopamine and non-dopamine transmission (Wise, 1978; Gallistel *et al.*, 1982; Spyraki *et al.*, 1982; Acquas *et al.*, 1989). Demonstrating that one neurotransmitter system is necessary and/or sufficient to produce a behavior does not eliminate all the other potential causes of that behavior. Creating a computational model would allow us to better understand the contributions and interactions of multiple neurochemical systems in a complex behavior such as priming.

3. Future Directions

To elucidate the primary neuronal mechanisms of the priming effect of rewards, we need to use techniques that allow for greater neuronal and temporal specificity than that provided by electrical brain stimulation and systemic dopamine receptor antagonism. Optogenetics would allow selective activation or silencing of neuronal circuits at specific time points. First, it should be examined whether optical activation of VTA-dopamine neurons produces a priming effect. Second, VTA dopamine should be silenced specifically when electrical priming stimulation is delivered. Those experiments would demonstrate whether VTA-dopamine transmission is sufficient and necessary to produce a priming effect.

Those optogenetic techniques could be used to investigate which non-dopamine circuits are involved in the priming effect. With the use of transgenic rodent lines, excitatory or inhibitory opsins can be expressed in non-dopamine neurons, such as orexin neurons. To assess the role of a specific projection, the opsin can be expressed in one brain region and the probe can be aimed at another brain region. For example, LH-orexin neurons can be transfected to express an opsin and an optical probe can be aimed at orexin terminals that project to the PVT. That method could be applied to the many other circuits implicated in motivated behavior such as

BLA-glutamate inputs to the NAc. Those circuits could be selectively activated during optical priming or silenced during electrical priming to reveal whether they are involved in the priming effect.

Lastly, the experiments conducted here studied the priming effect only in male rats. Historically, there has been a bias for studying the brain and behavior in male subjects. There have been misconceptions regarding the importance of considering sex differences (for review see Cahill, 2006; Beery & Zucker, 2011). More recently, there has been greater movement toward considering potential sex differences in behavioral neuroscience research. Although there is conflicting evidence that eICSS is affected by fluctuations in ovarian hormones (Woodside *et al.*, 1996; Bless *et al.*, 1997), there is evidence that operant responding for rewards such as food and alcohol depends on the rat estrus cycle (Roberts *et al.*, 1998; Richard *et al.*, 2017). Future priming studies that involve electrical brain stimulation, natural rewards, or drugs should be carried out on naturally cycling females or ovariectomized rats that receive estrogen or estrogen and progesterone replacement. It is important to consider sex differences in the priming effect because there are disorders that could be triggered by priming, such as binge eating, that are more prevalent in females (Udo & Grilo, 2018).

4. Conclusion

The priming effect of rewards can pose a serious problem for people that struggle with controlling food consumption or drug use. For example, food priming can lead to binge eating and drug priming can lead drug misuse. Elucidating the neurobiological mechanisms underlying the priming effect could help break the cycle of overconsuming food or misusing drugs.

Research on reward and motivation has largely implicated dopamine transmission. Yet, this thesis provides evidence consistent with the notion that certain aspects of motivation, such as the priming effect of rewards, may not depend on dopamine transmission. This highlights the importance of reconsidering the role of other, non-dopamine systems in reward and motivation.

References

Aberman, J.E. & Salamone, J.D. (1999) Nucleus accumbens dopamine depletions make rats more sensitive to high ratio requirements but do not impair primary food reinforcement. *Neuroscience*, **92**, 545–552.

Acquas, E., Carboni, E., Leone, P., & Chiara, D.G. (1989) SCH 23390 blocks drug-conditioned place-preference and place-aversion: Anhedonia (lack of reward) or apathy (lack of motivation) after dopamine-receptor blockade? *Psychopharmacology*, **99**, 151–155.

Ambroggi, F., Ishikawa, A., Fields, H.L., & Nicola, S.M. (2008) Basolateral amygdala neurons facilitate reward-seeking behavior by exciting nucleus accumbens neurons. *Neuron*, **59**, 648–661.

Anderson, R.M., Fatigati, M.D., & Rompre, P.-P. (1996) Estimates of the axonal refractory period of midbrain dopamine neurons: their relevance to brain stimulation reward. *Brain Res*, **718**, 83–88.

Arbuthnot, G. W., Crow, T. J., Fuxe, K., & Ungerstedt, U. (1970) Behavioral effects of stimulation in the region of the substantia nigra. *J Physiol*, **210**, 61P-62P.

Arvanitogiannis, A., Flores, C., Pfaus, J.G., & Shizgal, P. (1996) Increased ipsilateral expression of Fos following lateral hypothalamic self-stimulation. *Brain Res*, **720**, 148–154.

Arvanitogiannis, A. & Shizgal, P. (2008) The reinforcement mountain: Allocation of behavior as a function of the rate and intensity of rewarding brain stimulation. *Behav Neurosci*, **122**, 1126–1138.

Atalay, J. & Wise, R.A. (1983) Time course of pimozide effects on brain stimulation reward. *Pharmacol Biochem Behav*, **18**, 655–658.

Aston-Jones, G., Smith, R.J., Sartor, G.C., Moorman, D.E., Massi, L., Tahsili-Fahadan, P., & Richardson, K.A. (2010) Lateral hypothalamic orexin/hypocretin neurons: A role in reward-seeking and addiction. *Brain Res*, **1314**, 74–90.

Barbano, F.M. & Cador, M. (2005) Various aspects of feeding behavior can be partially dissociated in the rat by the incentive properties of food and the physiological state. *Behav Neurosci*, **119**, 1244–1253.

Bauco, P. & Wise, R.A. (1997) Synergistic effects of cocaine with lateral hypothalamic brain stimulation reward: lack of tolerance or sensitization. *J Pharmacol Exp Ther*, **283**, 1160–1167.

Beaulieu, J.-M. & Gainetdinov, R.R. (2011) The physiology, signaling, and pharmacology of dopamine receptors. *Pharmacol Rev*, **63**, 182–217.

Beierholm, U., Guitart-Masip, M., Economides, M., Chowdhury, R., Düzel, E., Dolan, R., & Dayan, P. (2013) Dopamine modulates reward-related vigor. *Neuropsychopharmacol*, **38**, 1495–1503.

Beckmann, J.S., Marusich, J.A., Gipson, C.D., & Bardo, M.T. (2011) Novelty seeking, incentive salience and acquisition of cocaine self-administration in the rat. *Behav Brain Res*, **216**, 159–165.

Beery, A.K. & Zucker, I. (2011) Sex bias in neuroscience and biomedical research. *Neurosci Biobehav Rev*, **35**, 565–572.

Beier, K.T., Steinberg, E.E., DeLoach, K.E., Xie, S., Miyamichi, K., Schwarz, L., Gao, X.J., Kremer, E.J., Malenka, R.C., & Luo, L. (2015) Circuit architecture of VTA dopamine neurons revealed by systematic input-output mapping. *Cell*, **162**, 622–634.

Beninger, R.J., Cheng, M., Hahn, B.L., Hoffman, D.C., Mazurski, E.J., Morency, M.A., Ramm, P., & Stewart, R.J. (1987) Effects of extinction, pimozide, SCH 23390, and metoclopramide on food-rewarded operant responding of rats. *Psychopharmacology*, **92**, 343–349.

Berridge, K.C. & Robinson, T.E. (1998) What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? *Brain Res Rev*, **28**, 309–369.

Berridge, K.C. & Robinson, T.E. (2003) Parsing reward. *Trends Neurosci*, **26**, 507–513.

Bielajew, C., Lapointe, M., Kiss, I., & Shizgal, P. (1982) Absolute and relative refractory periods of the substrates for lateral hypothalamic and ventral midbrain self-stimulation. *Physiol Behav*, **28**, 125–132.

Bielajew, C. & Shizgal, P. (1982) Behaviorally derived measures of conduction velocity in the substrate for rewarding medial forebrain bundle stimulation. *Brain Res*, **237**, 107–119.

Bigelow, G.E., Griffiths, R.R., & Liebson, I.A. (1977) Pharmacological influences upon human ethanol self-administration. *Alcohol Intoxication and Withdrawal—IIIb. Advances in Experimental Medicine and Biology,* **85B**, 523–538.

Bindra, D. (1969) A unified interpretation of emotion and motivation. *Ann Ny Acad Sci*, **159**, 1071–1083.

Bindra, D. (1978) How adaptive behavior is produced: A perceptual-motivational alternative to response-reinforcement. *The Behavioral and Brain Sciences*, **1**, 41–91.

Bless, E.P., McGinnis, K.A., Mitchell, A.L., Hartwell, A., & Mitchell, J.B. (1997) The effects of gonadal steroids on brain stimulation reward in female rats. *Behav Brain Res*, **82**, 235–244.

Bolles, R.C. (1962) The readiness to eat and drink: The effect of deprivation conditions. *J Comp Physiol Psych*, **55**, 230–234.

Bolles, R.C. & Moot, S.A. (1973) The rat's anticipation of two meals a day. *J Comp Physiol Psych*, **83**, 510–514.

Bonnavion, P., Mickelsen, L.E., Fujita, A., de Lecea, L., & Jackson, A.C. (2016) Hubs and spokes of the lateral hypothalamus: cell types, circuits and behaviour. *J Physiology*, **594**, 6443–6462.

Borgland, S.L., Chang, S.-J., Bowers, S.M., Thompson, J.L., Vittoz, N., Floresco, S.B., Chou, J., Chen, B.T., & Bonci, A. (2009) Orexin A/hypocretin-1 selectively promotes motivation for positive reinforcers. *J Neurosci*, **29**, 11215–11225.

Brown, P.L. & Jenkins, H.M. (1968) Auto-shaping of the pigeon's key-peck . *J Exp Anal Behav*, **11**, 1–8.

Breton, Y.-A., Mullett, A., Conover, K., & Shizgal, P. (2013) Validation and extension of the reward-mountain model. *Front Behav Neurosci*, **7**, 1–25.

Burstein, E., Ma, J., Wong, S., Gao, Y., Pham, E., Knapp, A., Nash, N., Olsson, R., Davis, R., Hacksell, U., Weiner, D., & Brann, M. (2005) Intrinsic efficacy of antipsychotics at human D2, D3, and D4 dopamine receptors: Identification of the clozapine metabolite N-Desmethylclozapine as a D2/D3 partial agonist. *J Pharmacol Exp Ther*, **315**, 1278–1287.

Cahill, L. (2006) Why sex matters for neuroscience. *Nat Rev Neurosci*, **7**, 477–484.

Chutuape, M.D., Mitchell, S.H., & de Wit, H. (1994) Ethanol preloads increase ethanol preference under concurrent random-ratio schedules in social drinkers. *Exp Clin Psychopharm*, **2**, 310–318.

Cofer, C.N. (1972) Motivation & Emotion. Glenview, Illinois: Scott, Foresman and Company.

Collier, G. & Levitsky, D. (1967) Defense of water balance in rats: Behavioral and physiological responses to depletion. *J Comp Physiol Psych*, **64**, 59–67.

Collier, G., Hirsch, E., & Kanarek, R. B. (1977) The operant revisited. In W.K. Honig & J. E. R. Staddon (Eds.) Handbook of operant behavior. Prentice-Hall: Englewood Cliffs, New Jersey.

Collins, L.E., Galtieri, D.J., Collins, P., Jones, S.K., Port, R.G., Paul, N.E., Hockemeyer, J., Müller, C.E., & Salamone, J.D. (2010) Interactions between adenosine and dopamine receptor antagonists with different selectivity profiles: Effects on locomotor activity. *Behav Brain Res*, **211**, 148–155.

Colombo, M. (2014) Deep and beautiful. The reward prediction error hypothesis of dopamine. *Stud Hist Philosophy Sci Part C Stud Hist Philosophy Biological Biomed Sci*, **45**, 57–67.

Corbett, D. & Wise, R.A. (1979) Intracranial self-stimulation in relation to the ascending noradrenergic fiber systems of the pontine tegmentum and caudal midbrain: A moveable electrode mapping study. *Brain Res*, **177**, 423–436.

Correa, M., Carlson, B.B., Wisniecki, A., & Salamone, J.D. (2002) Nucleus accumbens dopamine and work requirements on interval schedules. *Behav Brain Res*, **137**, 179–187.

Crow, T.J. (1969) Mode of enhancement of self stimulation in rats by methamphetamine. *Nature*, **224**, 709.

Crow, T.J. (1970) Enhancement by cocaine of intra-cranial self-stimulation in the rat. *Life Sci*, **9**, 375–381.

Crow, T.J. (1972) Catecholamine-containing neurones and electrical self-stimulation: 1. A review of some data. *Psychol Med*, **2**, 414–421.

Crow, T.J., Spear, P.J., & Arbuthnott, G.W. (1972) Intracranial self-stimulation with electrodes in the region of the locus coeruleus. *Brain Res*, **36**, 275–287.

Cousins, M.S., Atherton, A., Turner, L., & Salamone, J.D. (1996) Nucleus accumbens dopamine depletions alter relative response allocation in a T-maze cost/benefit task. *Behav Brain Res*, **74**, 189–197.

Daniela, E., Brennan, K., Gittings, D., Hely, L., & Schenk, S. (2004) Effect of SCH 23390 on (±)-3,4-methylenedioxymethamphetamine hyperactivity and self-administration in rats. *Pharmacol Biochem Be*, **77**, 745–750.

de Lecea, L., Kilduff, T.S., Peyron, C., Gao, X.-B., Danielson, P.E., Fukuhara, C., Battenberg, E.L.F., Gautvik, V.T., F.S., B.I., Frankel, W.N., van den Pol, A.N., Bloom, F.E., Gautvik,

K.M., & Sutcliffe, J.G. (1998) The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity. *Proc National Acad Sci*, **95**, 322–327.

de Wit, H. (1996) Priming effects with drugs and other reinforcers. *Exp Clin Psychopharm*, **4**, 5–10.

de Wit, H. & Stewart, J. (1981) Reinstatement of cocaine-reinforced responding in the rat. *Psychopharmacology*, **75**, 134–143.

de Wit, H. & Stewart, J. (1983) Drug reinstatement of heroin-reinforced responding in the rat. *Psychopharmacology*, **79**, 29–31.

Deluty, M.Z. (1976) Excitatory and inhibitory effects of free reinforcers. *Anim Learn Behav*, **4**, 436–440.

Deutsch, J.A., Adams, D.W., & Metzner, R.J. (1964) Choice of intracranial stimulation as a function of delay between stimulations and strength of competing drive. *J Comp Physiol Psych*, **57**, 241–243.

Di Ciano, P. & Everitt, B.J. (2004) Direct interactions between the basolateral amygdala and nucleus accumbens core underlie cocaine-seeking behavior by rats. *J Neurosci*, **24**, 7167–7173.

Dobi, A., Margolis, E.B., Wang, H.-L., Harvey, B.K., & Morales, M. (2010) Glutamatergic and nonglutamatergic neurons of the ventral tegmental area establish local synaptic contacts with dopaminergic and nondopaminergic neurons. *J Neurosci*, **30**, 218–229.

Drevets, W.C., Gautier, C., Price, J.C., Kupfer, D.J., Kinahan, P.E., Grace, A.A., Price, J.L., & Mathis, C.A. (2001) Amphetamine-induced dopamine release in human ventral striatum correlates with euphoria. *Biol Psychiat*, **49**, 81–96.

du Hoffmann, J. & Nicola, S.M. (2016) Activation of dopamine receptors in the nucleus accumbens promotes sucrose-reinforced cued approach behavior. *Front Behav Neurosci*, **10**, 1-19.

Edmonds, D.E. & Gallistel, C.R. (1974) Parametric analysis of brain stimulation reward in the rat: III. Effect of performance variables on the reward summation function. *J Comp Physiol Psych*, **87**, 876–883.

Edmonds, D.E. & Gallistel, C.R. (1977) Reward versus performance in self-stimulation: Electrode-specific effects of a-methyl-p-tyrosine on reward in the rat. *J Comp Physiol Psych*, **91**, 862–974.

Efron, B. & Tibshirani, R. (1986) Bootstrap methods for standard errors, confidence intervals, and other measures of statistical accuracy. *Stat Sci*, **1**, 54–75.

Eiserer, L.A. (1978) Effects of food primes on the operant behavior of nondeprived rats. *Anim Learn Behav*, **6**, 308–312.

Esposito, R.U., Motola, A.H.D., & Kornetsky, C. (1978) Cocaine: Acute effects of reinforcement thresholds for self-stimulation behavior to the medial forebrain bundle. *Pharmacol Biochem Behav*, **8**, 437–439.

Ettenberg, A. & Camp, C.H. (1986) Haloperidol induces a partial reinforcement extinction effect in rats: Implications for a dopamine involvement in food reward. *Pharmacol Biochem Be*, **25**, 813–821.

Evangelista, C., Hantson, A., Shams, W.M., Almey, A., Pileggi, M., Voisard, J.R., Boulos, V., Al- qadri, Y., Cautela, B.V., Zhou, F., Duchemin, J., Habrich, A., Tito, N., Koumrouyan, R.A., Patel, S., Lorenc, V., Gagne, C., Oufi, K., Shizgal, P., & Brake, W.G. (2019) The priming effect of food persists following blockade of dopamine receptors. *Eur J Neurosci*, **00,** 1–12.

Ewusi-Boisvert, E (2016). The effect of electrical priming stimulation on the reward-summation function. (Bachelor of Arts Honors Thesis). Concordia University, Montreal, Canada.

Fibiger, H., Carter, D., & Phillips, A. (1976) Decreased intracranial self-stimulation after neuroleptics or 6-hydroxydopamine: Evidence for mediation by motor deficits rather than by reduced reward. *Psychopharmacology*, **47**, 21–27.

Fibiger, H.C. (1978) Drugs and reinforcement mechanisms: a critical review of the catecholamine theory. *Annu Rev Pharmacol*, **18**, 37–56.

Fiorino, D.F., Coury, A., Fibiger, H.C., & Phillips, A.G. (1993) Electrical stimulation of reward sites in the ventral tegmental area increases dopamine transmission in the nucleus accumbens of the rat. *Behav Brain Res*, **55**, 131–141.

Flagel, S.B., Watson, S.J., Akil, H., & Robinson, T.E. (2008) Individual differences in the attribution of incentive salience to a reward-related cue: Influence on cocaine sensitization. *Behav Brain Res*, **186**, 48–56.

Fouriezos, G., Bielajew, C., & Pagotto, W. (1990) Task difficulty increases thresholds of rewarding brain stimulation. *Behav Brain Res*, **37**, 1–7.

Fouriezos, G. & Wise, R.A. (1976) Pimozide-induced extinction of intracranial self-stimulation: Response patterns rule out motor or performance deficits. *Brain Res*, **103**, 377–380.

Frank, R.A. & Williams, H.P. (1985) Both response effort and current intensity affect self-stimulation train duration thresholds. *Pharmacol Biochem Behav*, **22**, 527–530.

Franklin, K.B.J. (1978) Catecholamines and self-stimulation: Reward and performance effects dissociated. *Pharmacol Biochem Be*, **9**, 813–820.

Franklin, K.B.J. & McCoy, S.N. (1979) Pimozide-induced extinction in rats: Stimulus control of responding rules out motor deficit. *Pharmacol Biochem Behav*, **11**, 71–75.

Gallistel, C.R. (1966) Motivating effects in self-stimulation. *J Comp Physiol Psych*, **62**, 95–101.

Gallistel, C.R. (1969a) Self-stimulation: Failure of pretrial stimulation to affect rats' electrode preference. *J Comp Physiol Psych*, **69**, 722.

Gallistel, C.R. (1969b) The incentive of brain-stimulation reward. *J Comp Physiol Psych*, **69**, 713.

Gallistel, C.R., Boytim, M., Gomita, Y., & Klebanoff, L. (1982) Does pimozide block the reinforcing effect of brain stimulation? *Pharmacol Biochem Behav*, **17**, 769–781.

Gallistel, C.R. & Karras, D. (1984) Pimozide and amphetamine have opposing effects on the reward summation function. *Pharmacol Biochem Behav*, **20**, 73–77.

Gallistel, C.R., Shizgal, P., & Yeomans, J.S. (1981) A portrait of the substrate for self-stimulation. *Psychol Rev*, **88**, 228–273.

Gallistel, C.R., Stellar, J.R., & Bubis, E. (1974) Parametric analysis of brain stimulation reward in the rat: I. The transient process and the memory-containing process. *J Comp Physiol Psych*, **87**, 848–859.

Garrett, B.E. & Holtzman, S.G. (1994) D1 and D2 dopamine receptor antagonists block caffeine-induced stimulation of locomotor activity in rats. *Pharmacol Biochem Behav*, **47**, 89–94.

Geeraedts, L., Nieuwenhuys, R., & Veening, J. (1990a) Medial forebrain bundle of the rat: IV. Cytoarchitecture of the caudal (lateral hypothalamic) part of the medial forebrain bundle bed nucleus. *J Comp Neurol*, **294**, 537–568.

Geeraedts, L., Nieuwenhuys, R., & Veening, J. (1990b) Medial forebrain bundle of the rat: III. Cytoarchitecture of the rostral (telencephalic) part of the medial forebrain bundle bed nucleus. *J Comp Neurol*, **294**, 507–536.

German, D.C. & Bowden, D.M. (1974) Catecholamine systems as the neural substrate for intracranial self-stimulation: a hypothesis. *Brain Res*, **73**, 381–419.

Godfrey, N. & Borgland, S.L. (2019) Diversity in the lateral hypothalamic input to the ventral tegmental area. *Neuropharmacology*, **154**, 4–12.

Grace, A.A. (1991) Phasic versus tonic dopamine release and the modulation of dopamine system responsivity: A hypothesis for the etiology of schizophrenia. *Neuroscience*, **41**, 1–24.

Gratton, A. & Wise, R.A. (1983) Brain stimulation reward in the lateral hypothalamic medial forebrain bundle: Mapping of boundaries and homogeneity. *Brain Res*, **274**, 25–30.

Hall, H., Köhler, C., & Gawell, L. (1985) Some in vitro receptor binding properties of [3H]eticlopride, a novel substituted benzamide, selective for dopamine-D2 receptors in the rat brain. *Eur J Pharmacol*, **111**, 191–199.

Hamid, A.A., Pettibone, J.R., Mabrouk, O.S., Hetrick, V.L., Schmidt, R., Weele, C.M., Kennedy, R.T., Aragona, B.J., & Berke, J.D. (2015) Mesolimbic dopamine signals the value of work. *Nat Neurosci*, **19**, 117–126.

Harris, G.C., Wimmer, M., & Aston-Jones, G. (2005) A role for lateral hypothalamic orexin neurons in reward seeking. *Nature*, **437**, 556–559.

Hernandez, G., Breton, Y.-A., Conover, K., & Shizgal, P. (2010) At what stage of neural processing does cocaine act to boost pursuit of rewards? *Plos One*, **5**, 1–23.

Hernandez, G., Haines, E., Rajabi, H., Stewart, J., Arvanitogiannis, A., & Shizgal, P. (2007) Predictable and unpredictable rewards produce similar changes in dopamine tone. *Behav Neurosci*, **121**, 887–895.

Hernandez, G., Hamdani, S., Rajabi, H., Conover, K., Stewart, J., Arvanitogiannis, A., & Shizgal, P. (2006) Prolonged rewarding stimulation of the rat medial forebrain bundle: Neurochemical and behavioral consequences. *Behav Neurosci*, **120**, 888–904.

Hernandez, G., Trujillo-Pisanty, I., Cossette, M.-P., Conover, K., & Shizgal, P. (2012) Role of dopamine tone in the pursuit of brain stimulation reward. *J Neurosci*, **32**, 11032–11041.

Hoebel, B.G., Monaco, A.P., Hernandez, L., Aulisi, E.F., Stanley, G.B., & Lenard, L. (1983) Self-injection of amphetamine directly into the brain. *Psychopharmacology*, **81**, 158–163.

Hoebel, B.G. & Teitelbaum, P. (1962) Hypothalamic control of feeding and self-stimulation. *Science*, **135**, 375–377.

Hull, C. L. (1943) Principles of behavior: An introduction to behavior theory. Oxford, England: Appleton-Century.

Ikemoto, S. (2007) Dopamine reward circuitry: Two projection systems from the ventral midbrain to the nucleus accumbens–olfactory tubercle complex. *Brain Res Rev*, **56**, 27–78.

Ikemoto, S. & Panksepp, J. (1999) The role of nucleus accumbens dopamine in motivated behavior: A unifying interpretation with special reference to reward-seeking. *Brain Res Rev*, **31**, 6–41.

Inglis, I., Forkman, B., & Lazarus, J. (1997) Free food or earned food? A review and fuzzy model of contrafreeloading. *Anim Behav*, **53**, 1171–1191.

Jensen, G.D. (1963) Preference for bar pressing over "freeloading" as a function of number of rewarded presses. *J Exp Psychol*, **65**, 451–454.

Kadar, E., Aldavert- Vera, L., Huguet, G., Costa- Miserachs, D., Morgado- Bernal, I., & Segura-Torres, P. (2011) Intracranial self- stimulation induces expression of learning and memory- related genes in rat amygdala. *Genes Brain Behav*, **10**, 69–77.

Kampov-Polevoy, A.B., Alterman, A., Khalitov, E., & Garbutt, J.C. (2006) Sweet preference predicts mood altering effect of and impaired control over eating sweet foods. *Eat Behav*, **7**, 181–187.

Kimble, G.A. (1951) Behavior strength as a function of the intensity of the hunger drive. *J Exp Psychol*, **41**, 341–348.

Kirk, J.M. & de Wit, H. (2000) Individual differences in the priming effect of ethanol in social drinkers. *J Stud Alcohol*, **61**, 64–71.

Koob, G.F., Fray, P.J., & Iversen, S.D. (1978) Self-stimulation at the lateral hypothalamus and locus coeruleus after specific unilateral lesions of the dopamine system. *Brain Res*, **146**, 123–140.

Kroeze, W.K., Hufeisen, S.J., Popadak, B.A., Renock, S.M., Steinberg, S., Ernsberger, P., Jayathilake, K., Meltzer, H.Y., & Roth, B.L. (2003) H1-histamine receptor affinity predicts short-term weight gain for typical and atypical antipsychotic drugs. *Neuropsychopharmacol*, **28**, 519-526.

Lamb, R.J., Preston, K.L., Schindler, C.W., Meisch, R.A., Davis, F., Katz, J.L., Henningfield, J.E., & Goldberg, S.R. (1991) The reinforcing and subjective effects of morphine in post-addicts: A dose-response study. *J Pharmacol Exp Ther*, **259**, 1165–1173.

Lammel, S., Lim, B., & Malenka, R.C. (2014) Reward and aversion in a heterogeneous midbrain dopamine system. *Neuropharmacology*, **76**, 351–359.

Lammel, S., Lim, B., Ran, C., Huang, K., Betley, M.J., Tye, K.M., Deisseroth, K., & Malenka, R.C. (2012) Input-specific control of reward and aversion in the ventral tegmental area. *Nature*, **491**, 212–217.

Lazenka, M.F., Legakis, L.P., & Negus, S.S. (2016) Opposing effects of dopamine D1- and D2-like agonists on intracranial self-stimulation in male rats. *Exp Clin Psychopharm*, **24**, 193–205.

Leri, F. & Stewart, J. (2001) Drug-induced reinstatement to heroin and cocaine seeking: A rodent model of relapse in polydrug use. *Exp Clin Psychopharm*, **9**, 297–306.

Levy, D.I. & Sinnamon, H.M. (1990) Midbrain areas required for locomotion initiated by electrical stimulation of the lateral hypothalamus in the anesthetized rat. *Neuroscience*, **39**, 665–674.

Liu, S., Globa, A.K., Mills, F., Naef, L., Qiao, M., Bamji, S.X., & Borgland, S.L. (2016) Consumption of palatable food primes food approach behavior by rapidly increasing synaptic density in the VTA. *Proc National Acad Sci*, **113**, 2520–2525.

Lowe, M.R. & Butryn, M.L. (2007) Hedonic hunger: A new dimension of appetite? *Physiol Behav*, **91**, 432–439.

Ludwig, A.M. & Wikler, A. (1974) "Craving" and relapse to drink. *Q J Stud Alcohol*, **35**, 108–130.

Margules, D.L. & Olds, J. (1962) Identical "feeding" and "rewarding" systems in the lateral hypothalamus of rats. *Science*, **135**, 374–375.

Martelle, J.L. & Nader, M.A. (2008) A review of the discovery, pharmacological characterization, and behavioral effects of the dopamine D2-like receptor antagonist eticlopride. *Cns Neurosci Ther*, **14**, 248–262.

Matsumoto, M. & Hikosaka, O. (2007) Lateral habenula as a source of negative reward signals in dopamine neurons. *Nature*, **447**, 1111.

McMullen, N.T. & Almli, R.C. (1981) Cell types within the medial forebrain bundle: A golgi study of preoptic and hypothalamic neurons in the rat. *Am J Anat*, **161**, 323–340.

Melis, M. & Argiolas, A. (1995) Dopamine and sexual behavior. *Neurosci Biobehav Rev*, **19**, 19–38.

Mestikawy, S., Wallén-Mackenzie, Å., Fortin, G.M., Descarries, L., & Trudeau, L.-E. (2011) From glutamate co-release to vesicular synergy: Vesicular glutamate transporters. *Nat Rev Neurosci*, **12**, 204–216.

Meyer, P.J., Lovic, V., Saunders, B.T., Yager, L.M., Flagel, S.B., Morrow, J.D., & Robinson, T.E. (2012) Quantifying individual variation in the propensity to attribute incentive salience to reward cues. *Plos One*, **7**, 1–15.

Mickelsen, L.E., Bolisetty, M., Chimileski, B.R., Fujita, A., Beltrami, E.J., Costanzo, J.T., Naparstek, J.R., Robson, P., & Jackson, A.C. (2019) Single-cell transcriptomic analysis of the lateral hypothalamic area reveals molecularly distinct populations of inhibitory and excitatory neurons. *Nat Neurosci*, **22**, 642–656.

Miliaressis, E., Rompre, P.-P., Laviolette, P., Philippe, L., & Coulombe, D. (1986) The curve-shift paradigm in self-stimulation. *Physiol Behav*, **37**, 85–91.

Mingote, S., Weber, S.M., Ishiwari, K., Correa, M., & Salamone, J.D. (2005) Ratio and time requirements on operant schedules: Effort- related effects of nucleus accumbens dopamine depletions. *Eur J Neurosci*, **21**, 1749–1757.

Mohebi, A., Pettibone, J.R., Hamid, A.A., Wong, J.-M.T., Vinson, L.T., Patriarchi, T., Tian, L., Kennedy, R.T., & Berke, J.D. (2019) Dissociable dopamine dynamics for learning and motivation. *Nature*, 1–6.

Montague, P.R., Dayan, P., & Sejnowski, T.J. (1996) A framework for mesencephalic dopamine systems based on predictive Hebbian learning. *J Neurosci*, **16**, 1936–1947.

Morales, M. & Margolis, E.B. (2017) Ventral tegmental area: Cellular heterogeneity, connectivity and behaviour. *Nat Rev Neurosci*, **18**, 73–85.

Morales, M. & Root, D.H. (2014) Glutamate neurons within the midbrain dopamine regions. *Neuroscience*, **282**, 60–68.

Morgan, C.T. & Fields, P.E. (1938) The effect of variable preliminary feeding upon the rat's speed-of-locomotion. *J Comp Psychol*, **26**, 331–348.

Murray, B. & Shizgal, P. (1994) Evidence implicating both slow- and fast-conducting fibers in the rewarding effect of medial forebrain bundle stimulation. *Behav Brain Res*, **63**, 47–60.

Murray, B. & Shizgal, P. (1996a) Behavioral measures of conduction velocity and refractory period for reward-relevant axons in the anterior LH and VTA. *Physiol Behav*, **59**, 643–652.

Murray, B. & Shizgal, P. (1996b) Physiological measures of conduction velocity and refractory period for putative reward-relevant MFB axons arising in the rostral MFB. *Physiol Behav*, **59**, 427–437.

Nair-Roberts, R.G., Chatelain-Badie, S.D., Benson, E., White-Cooper, H., Bolam, J.P., & Ungless, M.A. (2008) Stereological estimates of dopaminergic, GABAergic and glutamatergic neurons in the ventral tegmental area, substantia nigra and retrorubral field in the rat. *Neuroscience*, **152**, 1024–1031.

Nieuwenhuys, R., Geeraedts, L.M., & Veening, J.G. (1982) The medial forebrain bundle of the rat. I. General introduction. *J Comp Neurol*, **206**, 49–81.

Niv, Y. (2007) Cost, benefit, tonic, phasic: What do response rates tell us about dopamine and motivation? *Ann Ny Acad Sci*, **1104**, 357–376.

Niv, Y., Daw, N., & Dayan, P. (2005) How fast to work: Response vigor, motivation, and tonic dopamine, *Adv in Neur Info Process Syst*, 1019–1026.

Niv, Y., Daw, N.D., Joel, D., & Dayan, P. (2007) Tonic dopamine: Opportunity costs and the control of response vigor. *Psychopharmacology*, **191**, 507–520.

Niv, Y., Joel, D., & Dayan, P. (2006) A normative perspective on motivation. *Trends Cogn Sci*, **10**, 375–381.

Nolan, S.A. & Heinzen, T.E. (2011) Essentials of statistics for the behavioral sciences. New York, New York: Worth Publishers.

Olds, J. (1956) A preliminary mapping of electrical reinforcing effects in the rat brain. *J Comp Physiol Psych*, **49**, 281–285.

Olds, J. & Milner, P. (1954) Positive reinforcement produced by electrical stimulation of septal area and other regions of rat brain. *J Comp Physiol Psych*, **47**, 419–427.

Olds, M.E. & Olds, J. (1963) Approach- avoidance analysis of rat diencephalon. *J Comp Neurol*, **120**, 259–295.

Osborne, S.R. (1977) The free food (contrafreeloading) phenomenon: A review and analysis. *Anim Learn Behav*, **5**, 221–235.

Paxinos, G. & Watson, C. (2007) The rat brain in stereotaxic coordinates (6 ed.), Academic Press.

Peciña, S., Berridge, K.C., & Parker, L.A. (1997) Pimozide does not shift palatability: Separation of anhedonia from sensorimotor suppression by taste reactivity. *Pharmacol Biochem Behav*, **58**, 801–811.

Pezze, M.A., Marshall, H.J., & Cassaday, H.J. (2015) Dopaminergic modulation of appetitive trace conditioning: The role of D1 receptors in medial prefrontal cortex. *Psychopharmacology*, **232**, 2669–2680.

Phillips, A.G. (1984) Brain reward circuitry: A case for separate systems. *Brain Res Bull*, **12**, 195–201.

Phillips, P.E., Stuber, G.D., Helen, M.L., Wightman, M.R., & Carelli, R.M. (2003) Subsecond dopamine release promotes cocaine seeking. *Nature*, **422**, 614–617.

Piazza, P. & Le Moal, M. (1996) Pathophysiological basis of vulnerability to drug abuse: Role of an interaction between stress, glucocorticoids, and dopaminergic neurons. *Annu Rev Pharmacol*, **36**, 359–378.

Piazza, P.V., Deminière, J.M., Le Moal, M., & Simon, H. (1989) Factors that predict individual vulnerability to amphetamine self-administration. *Science*, **245**, 1511–1513.

Piazza, P.V., Maccari, S., Deminière, J.-M., Le Moal, M., Mormède, P., & Simon, H. (1991) Corticosterone levels determine individual vulnerability to amphetamine self-administration. *Proc National Acad Sci*, **88**, 2088–2092.

Poller, W.C., Madai, V.I., Bernard, R., Laube, G., & Veh, R.W. (2013) A glutamatergic projection from the lateral hypothalamus targets VTA-projecting neurons in the lateral habenula of the rat. *Brain Res*, **1507**, 45–60.

Poschel, B.P.H. & Ninteman, F.W. (1963) Norepinephrine: A possible excitatory neurohormone of the reward system. *Life Sci*, **2**, 782–788.

Poulin, J.-F., Caronia, G., Hofer, C., Cui, Q., Helm, B., Ramakrishnan, C., Chan, S.C., Dombeck, D.A., Deisseroth, K., & Awatramani, R. (2018) Mapping projections of molecularly defined dopamine neuron subtypes using intersectional genetic approaches. *Nat Neurosci*, **21**, 1260–1271.

Pritzel, M., Huston, J.P., & Buscher, W. (1983) Hypothalamic self-stimulation in rats with one hemisphere isolated anterior to the midbrain and the other hemisphere devoid of the telencephalon. *Exp Neurol*, **81**, 426–445.

Qi, J., Zhang, S., Wang, H.-L., Barker, D.J., Miranda-Barrientos, J., & Morales, M. (2016) VTA glutamatergic inputs to nucleus accumbens drive aversion by acting on GABAergic interneurons. *Nat Neurosci*, **19**, 725–733.

Randall, P.A., Lee, C.A., Podurgiel, S.J., Hart, E., Yohn, S.E., Jones, M., Rowland, M., López-Cruz, L., Correa, M., & Salamone, J.D. (2015) Bupropion increases selection of high effort activity in rats tested on a progressive ratio/chow feeding choice procedure: Implications for treatment of effort-related motivational symptoms. *Int J Neuropsychoph*, **18**, 1–11.

Reid, L.D., Hunsicker, J.P., Kent, E.W., Lindsay, J.L., & Gallistel, C.R. (1973) Incidence and magnitude of the "priming effect" in self-stimulating rats. *J Comp Physiol Psych*, **82**, 286–293.

Ren, S., Wang, Y., Yue, F., Cheng, X., Dang, R., Qiao, Q., Sun, X., Li, X., Jiang, Q., Yao, J., Qin, H., Wang, G., Liao, X., Gao, D., Xia, J., Zhang, J., Hu, B., Yan, J., Wang, Y., Xu, M., Han, Y., Tang, X., Chen, X., He, C., & Hu, Z. (2018) The paraventricular thalamus is a critical thalamic area for wakefulness. *Science*, **362**, 429–434.

Richard, J.E., López-Ferreras, L., Anderberg, R.H., Olandersson, K., & Skibicka, K.P. (2017) Estradiol is a critical regulator of food-reward behavior. *Psychoneuroendocrino*, **78**, 193–202.

Richelson, E. & Souder, T. (2000) Binding of antipsychotic drugs to human brain receptors Focus on newer generation compounds. *Life Sci*, **68**, 29–39.

Roberts, A.J., Smith, A.D., Weiss, F., Rivier, C., & Koob, G.F. (1998) Estrous cycle effects on operant responding for ethanol in female rats. *Alcohol Clin Exp Res*, **22**, 1564–1569.

Robinson, M., Robinson, T.E., & Berridge, K.C. (2013) Incentive salience and the transition to addiction. *Biol Res Addict*, **2**, 391–399.

Robinson, T.E. & Berridge, K.C. (1993) The neural basis of drug craving: An incentive-sensitization theory of addiction. *Brain Res Rev*, **18**, 247–291.

Rodeberg, N.T., Johnson, J.A., Bucher, E.S., & Wightman, M.R. (2016) Dopamine dynamics during continuous intracranial self-stimulation: Effect of waveform on fast-scan cyclic voltammetry data. *ACS Chem Neurosci*, **7**, 1508–1518.

Roeper, J. (2013) Dissecting the diversity of midbrain dopamine neurons. *Trends Neurosci*, **36**, 336–342.

Roitman, M.F., Stuber, G.D., Phillips, P.E., Wightman, M.R., & Carelli, R.M. (2004) Dopamine operates as a subsecond modulator of food seeking. *J Neurosci*, **24**, 1265–1271.

Rolls, E.T. (1972) Activation of amygdaloid neurones in reward, eating and drinking elicited by electrical stimulation of the brain. *Brain Res*, **45**, 365–381.

Root, D.H., Estrin, D.J., & Morales, M. (2018) Aversion or salience signaling by ventral tegmental area glutamate neurons. *iScience*, **2**, 51–62.

Sakurai, T., Amemiya, A., Ishii, M., Matsuzaki, I., Chemelli, R.M., Tanaka, H., Williams, C.S., Richardson, J.A., Kozlowski, G.P., Wilson, S., Arch, J.R., Buckingham, R.E., Haynes, A.C., Carr, S.A., Annan, R.S., McNulty, D.E., Liu, W.-S., Terrett, J.A., Elshourbagy, N.A., Bergsma, D.J., & Yanagisawa, M. (1998) Orexins and orexin receptors: A family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding Behavior. *Cell*, **92**, 573–585.

Salamone, J.D. & Correa, M. (2002) Motivational views of reinforcement: Implications for understanding the behavioral functions of nucleus accumbens dopamine. *Behav Brain Res*, **137**, 3–25.

Salamone, J.D. & Correa, M. (2012) The mysterious motivational functions of mesolimbic dopamine. *Neuron*, **76**, 470–485.

Salamone, J.D., Correa, M., Farrar, A., & Mingote, S.M. (2007) Effort-related functions of nucleus accumbens dopamine and associated forebrain circuits. *Psychopharmacology*, **191**, 461–482.

Salamone, J.D., Correa, M., Farrar, A.M., Nunes, E.J., & Pardo, M. (2009) Dopamine, behavioral economics, and effort. *Front Behav Neurosci*, **3**, 1–12.

Salamone, J.D., Correa, M., Mingote, S., & Webber, S.M. (2003) Nucleus accumbens dopamine and the regulation of effort in food-seeking behavior: Implications for studies of natural motivation, psychiatry, and drug abuse. *J Pharmacol Exp Ther*, **305**, 1–8.

Salamone, J.D., Correa, M., Mingote, S.M., & Weber, S.M. (2005) Beyond the reward hypothesis: Alternative functions of nucleus accumbens dopamine. *Curr Opin Pharmacol*, **5**, 34–41.

Salamone, J.D., Correa, M., Yohn, S., Cruz, L., Miguel, N., & Alatorre, L. (2016) The pharmacology of effort-related choice behavior: Dopamine, depression, and individual differences. *Behav Process*, **127**, 3–17.

Salamone, J.D., Cousins, & Snyder, B.J. (1997) Behavioral functions of nucleus accumbens dopamine: Empirical and conceptual problems with the anhedonia hypothesis. *Neurosci Biobehav Rev*, **21**, 341–359.

Salamone, J.D., Wisniecki, A., Carlson, B.B., & Correa, M. (2001) Nucleus accumbens dopamine depletions make animals highly sensitive to high fixed ratio requirements but do not impair primary food reinforcement. *Neuroscience*, **105**, 863–870.

Schultz, W. (2015) Neuronal reward and decision signals: From theories to data. *Physiol Rev*, **95**, 853–951.

Schultz, W. (2016) Dopamine reward prediction-error signalling: A two-component response. *Nat Rev Neurosci*, **17**, 183–195.

Schultz, W., Dayan, P., & Montague, R.P. (1997) A neural substrate of prediction and reward. *Science*, **275**, 1593–1599.

Self, D.W. & Nestler, E.J. (1998) Relapse to drug-seeking: Neural and molecular mechanisms. *Drug Alcohol Depen*, **51**, 49–60.

Shaham, Y., Shalev, U., Lu, L., de Wit, H., & Stewart, J. (2003) The reinstatement model of drug relapse: History, methodology and major findings. *Psychopharmacology*, **168**, 3–20.

Shizgal, P. (1989) Toward a cellular analysis of intracranial self-stimulation: Contributions of collision studies. *Neurosci Biobehav Rev*, **13**, 81–90.

Shizgal, P., Bielajew, C., Corbett, D., Skelton, R., & Yeomans, J. (1980) Behavioral methods for inferring anatomical linkage between rewarding brain stimulation sites. *J Comp Physiol Psych*, **94**, 227–237.

Siegal, P.S. (1961) Food intake in the rat in relation to the dark-light cycle. *J Comp Physiol Psych*, **54**, 294–301.

Sinnamon, H.M., Ginzburg, R.N., & Kurose, G.A. (1987) Midbrain stimulation in the anesthetized rat: Direct locomotor effects and modulation of locomotion produced by hypothalamic stimulation. *Neuroscience*, **20**, 695–707.

Sinnamon, H.M. & Sklow, B. (1990) Latency to initiate locomotion elicited by stimulation of the diencephalon positively correlates in awake and anesthetized rats. *Pharmacol Biochem Behav*, **36**, 725–728.

Solomon, R.B., Trujillo-Pisanty, I., Conover, K., & Shizgal, P. (2015) Psychophysical inference of frequency-following fidelity in the neural substrate for brain stimulation reward. *Behav Brain Res*, **292**, 327–341.

Spyraki, C., Fibiger, H.C., & Phillips, A.G. (1982) Dopaminergic substrates of amphetamine-induced place preference conditioning. *Brain Res*, **253**, 185–193.

Stein, L. (1968) Chemistry of reward and punishment. In: Proceedings of the American College of Neuropsychopharmacology, Washington, DC: U.S. Government Printing Office.

Steinberg, E.E., Boivin, J.R., Saunders, B.T., Witten, I.B., Deisseroth, K., & Janak, P.H. (2014) Positive reinforcement mediated by midbrain dopamine neurons requires D1 and D2 receptor activation in the nucleus accumbens. *Plos One*, **9**, 1–9.

Steiner, J.E., Glaser, D., Hawilo, M.E., & Berridge, K.C. (2001) Comparative expression of hedonic impact: Affective reactions to taste by human infants and other primates. *Neurosci Biobehav Rev*, **25**, 53–74.

Stellar, J.R. & Gallistel, C.R. (1975) Runway performance of rats for brain-stimulation or food reward: Effects of hunger and priming. *J Comp Physiol Psych*, **89**, 590–599.

Stockwell, T.R., Hodgson, R.J., Rankin, H.J., & Taylor, C. (1982) Alcohol dependence, beliefs and the priming effect. *Behav Res Ther*, **20**, 513–522.

Stuber, G.D., Hnasko, T.S., Britt, J.P., Edwards, R.H., & Bonci, A. (2010) Dopaminergic terminals in the nucleus accumbens but not the dorsal striatum corelease glutamate. *J Neurosci*, **30**, 8229–8233.

Stuber, G.D., Sparta, D.R., Stamatakis, A.M., van Leeuwen, W.A., Hardjoprajitno, J.E., Cho, S., Tye, K.M., Kempadoo, K.A., Zhang, F., Deisseroth, K., & Bonci, A. (2011) Excitatory transmission from the amygdala to nucleus accumbens facilitates reward seeking. *Nature*, **475**, 377–381.

Stuber, G.D. & Wise, R.A. (2016) Lateral hypothalamic circuits for feeding and reward. *Nat Neurosci*, **19**, 198–205.

Substance Abuse and Mental Health Services Administration. (2017) National Survey on Drug Use and Health. Retrieved from https://www.samhsa.gov/data/sites/default/files/cbhsq-reports/NSDUHDetailedTabs2017/NSDUHDetailedTabs2017.htm#tab2-33A.

Swanson, L.W. (1982) The projections of the ventral tegmental area and adjacent regions: A combined fluorescent retrograde tracer and immunofluorescence study in the rat. *Brain Res Bull*, **9**, 321–353.

Terry, W. (1980) Effects of food-reinforcer primes during acquisition of an instrumental running response in rats. *J Gen Psychology*, **102**, 265–274.

Terry, W. (1983) Effects of food priming on instrumental acquisition and performance. *Learn Motiv*, **14**, 107–122.

Toates, F.M. (1981) The control of ingestive behaviour by internal and external stimuli—A theoretical review. *Appetite*, **2**, 35–50.

Toates, F.M. (1986) Motivational Systems. Cambridge, United Kingdom: Cambridge University Press.

Tomie, A., Aguado, A.S., Poherecky, L.A., & Benjamin, D. (2000) Individual differences in pavlovian autoshaping of lever pressing in rats predict stress-induced corticosterone release and mesolimbic levels of monoamines. *Pharmacol Biochem Behav*, **65**, 509–517.

Trujillo-Pisanty, I., Conover, K., & Shizgal, P. (2014) A new view of the effect of dopamine receptor antagonism on operant performance for rewarding brain stimulation in the rat. *Psychopharmacology*, **231**, 1351–1364.

Udo, T. & Grilo, C.M. (2018) Prevalence and correlates of DSM-5–defined eating disorders in a nationally representative sample of U.S. adults. *Biol Psychiat*, **84**, 345–354.

Uslaner, J.M., Acerbo, M.J., Jones, S.A., & Robinson, T.E. (2006) The attribution of incentive salience to a stimulus that signals an intravenous injection of cocaine. *Behav Brain Res*, **169**, 320–324.

van der Kooy, D. & Hogan, J.A. (1978) Priming effects with food and water reinforcers in hamsters. *Learn Motiv*, **9**, 332–346.

Volkow, N.D., Wang, G.-J., Fowler, J.S., Logan, J., Gatley, S.J., Wong, C., Hitzemann, R., & Pappas, N.R. (1999) Reinforcing effects of psychostimulants in humans are associated

with increases in brain dopamine and occupancy of D(2) receptors. *J Pharmacol Exp Ther*, **291**, 409–415.

Walton, M.E. & Bouret, S. (2019) What Is the Relationship between Dopamine and Effort? *Trends Neurosci*, **42**, 79–91.

Wang, D.V., Viereckel, T., Zell, V., Konradsson-Geuken, Å., Broker, C.J., Talishinsky, A., Yoo, J., Galinato, M.H., Arvidsson, E., Kesner, A.J., Hnasko, T.S., Wallén-Mackenzie, Å., & Ikemoto, S. (2017) Disrupting glutamate co-transmission does not affect acquisition of conditioned behavior reinforced by dopamine neuron activation. *Cell Reports*, **18**, 2584–2591.

Wang, R.Y. (1981) Dopaminergic neurons in the rat ventral tegmental area. I. Identification and characterization. *Brain Res Rev*, **3**, 123–140.

Wasserman, E.M., Gomita, Y., & Gallistel, C.R. (1982) Pimozide blocks reinforcement but not priming from MFB stimulation in the rat. *Pharmacol Biochem Behav*, **17**, 783–787.

White, N.M. (1989) Reward or reinforcement: What's the difference? *Neurosci Biobehav Rev*, **13**, 181–186.

Williams, D.R. & Williams, H. (1969) Auto-maintenance in the pigeon: Sustained pecking despite contingent non-reinforcement. *J Exp Anal Behav*, **12**, 511–520.

Wise, R.A. (1978) Catecholamine theories of reward: A critical review. *Brain Res*, **152**, 215–247.

Wise, R.A. (1980) Action of drugs of abuse on brain reward systems. *Pharmacol Biochem Behav*, **13**, 213–223.

Wise, R.A. (1982) Neuroleptics and operant behavior: The anhedonia hypothesis. *Behav Brain Sci*, **5**, 39–53.

Wise, R.A. (1998) Drug-activation of brain reward pathways. *Drug Alcohol Depen*, **51**, 13–22.

Wise, R.A. (1996) Addictive drugs and brain stimulation reward. *Annu Rev Neurosci*, **19**, 319–340.

Wise, R.A. (2004) Dopamine, learning and motivation. *Nat Rev Neurosci*, **5**, 1–12.

Wise, R.A. (2006) Role of brain dopamine in food reward and reinforcement. *Philosophical Transactions Royal Soc B Biological Sci*, **361**, 1149–1158.

Wise, R.A. (2008) Dopamine and reward: The anhedonia hypothesis 30 years on. *Neurotox Res*, **14**, 169–183.

Wise, R.A., Murray, A., & Bozarth, M.A. (1990) Bromocriptine self-administration and bromocriptine-reinstatement of cocaine-trained and heroin-trained lever pressing in rats. *Psychopharmacology*, **100**, 355–360.

Wise, R.A. & Rompre, P.-P. (1989) Brain dopamine and reward. *Annu Rev Psychol*, **40**, 191–25.

Wise, R.A. & Schwartz, H.V. (1981) Pimozide attenuates acquisition of lever-pressing for food in rats. *Pharmacol Biochem Be*, **15**, 655–656.

Wise, R.A., Spindler, J., de Wit, H., & Gerber, G.J. (1978) Neuroleptic-induced "anhedonia" in rats: Pimozide blocks reward quality of food. *Science*, **201**, 262–264.

Witten, I.B., Steinberg, E.E., Lee, S., Davidson, T.J., Zalocusky, K.A., Brodsky, M., Yizhar, O., Cho, S.L., Gong, S., Ramakrishnan, C., Stuber, G.D., Tye, K.M., Janak, P.H., & Deisseroth, K. (2011) Recombinase-driver rat lines: Tools, techniques, and optogenetic application to dopamine-mediated reinforcement. *Neuron*, **72**, 721–733.

Woodside, B., Renaudin, A., & Shizgal, P. (1996) Administration of ovarian steroid hormones does not change the reward effectiveness of lateral hypothalamic stimulation in ovariectomized rats. *Psychobiology*, **24**, 202–210.

Wyvell, C.L. & Berridge, K.C. (2000) Intra-accumbens amphetamine increases the conditioned incentive salience of sucrose reward: Enhancement of reward "wanting" without enhanced "liking" or response reinforcement. *J Neurosci*, **20**, 8122–8130.

Yamaguchi, T., Sheen, W., & Morales, M. (2007) Glutamatergic neurons are present in the rat ventral tegmental area. *Eur J Neurosci*, **25**, 106–118.

Yamaguchi, T., Wang, H.-L., Li, X., Ng, T.H., & Morales, M. (2011) Mesocorticolimbic glutamatergic pathway. *J Neurosci*, **31**, 8476–8490.

Yang, Y., Wang, H., Hu, J., & Hu, H. (2018) Lateral habenula in the pathophysiology of depression. *Curr Opin Neurobiol*, **48**, 90–96.

Yeomans, J.S. (1979) The absolute refractory periods of self-stimulation neurons. *Physiol Behav*, **22**, 911–919.

Yeomans, J.S. (1989) Two substrates for medial forebrain bundle self-stimulation: Myelinated axons and dopamine axons. *Neurosci Biobehav Rev*, **13**, 91–98.

Yeomans, J.S., Kofman, O., & McFarlane, V. (1985) Cholinergic involvement in lateral hypothalamic rewarding brain stimulation. *Brain Res*, **329**, 19–26.

Yeomans, J.S., Maidment, N.T., & Bunney, B.S. (1988) Excitability properties of medial forebrain bundle axons of A9 and A10 dopamine cells. *Brain Res*, **450**, 86–93.

Yoo, J., Zell, V., Gutierrez-Reed, N., Wu, J., Ressler, R., Shenasa, M., Johnson, A.B., Fife, K.H., Faget, L., & Hnasko, T.S. (2016) Ventral tegmental area glutamate neurons co-release GABA and promote positive reinforcement. *Nat Commun*, **7**, 1–13.

You, Z.-B., Chen, Y.-Q., & Wise, R.A. (2001) Dopamine and glutamate release in the nucleus accumbens and ventral tegmental area of rat following lateral hypothalamic self-stimulation. *Neuroscience*, **107**, 629–639.

Young, P. (1959) The role of affective processes in learning and motivation. *Psychol Rev*, **66**, 104–125.